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THE LIVING UNIVERSE

An Encyclopaedia of
the Biological Sciences

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THE LIVING UNIVERSE : AN ENCYCLOPAEDIA OF THE BIOLOGICAL SCIENCES

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An Encyclopaedia of the Biological Sciences

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L'ÊTRE VIVANT
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The living organism

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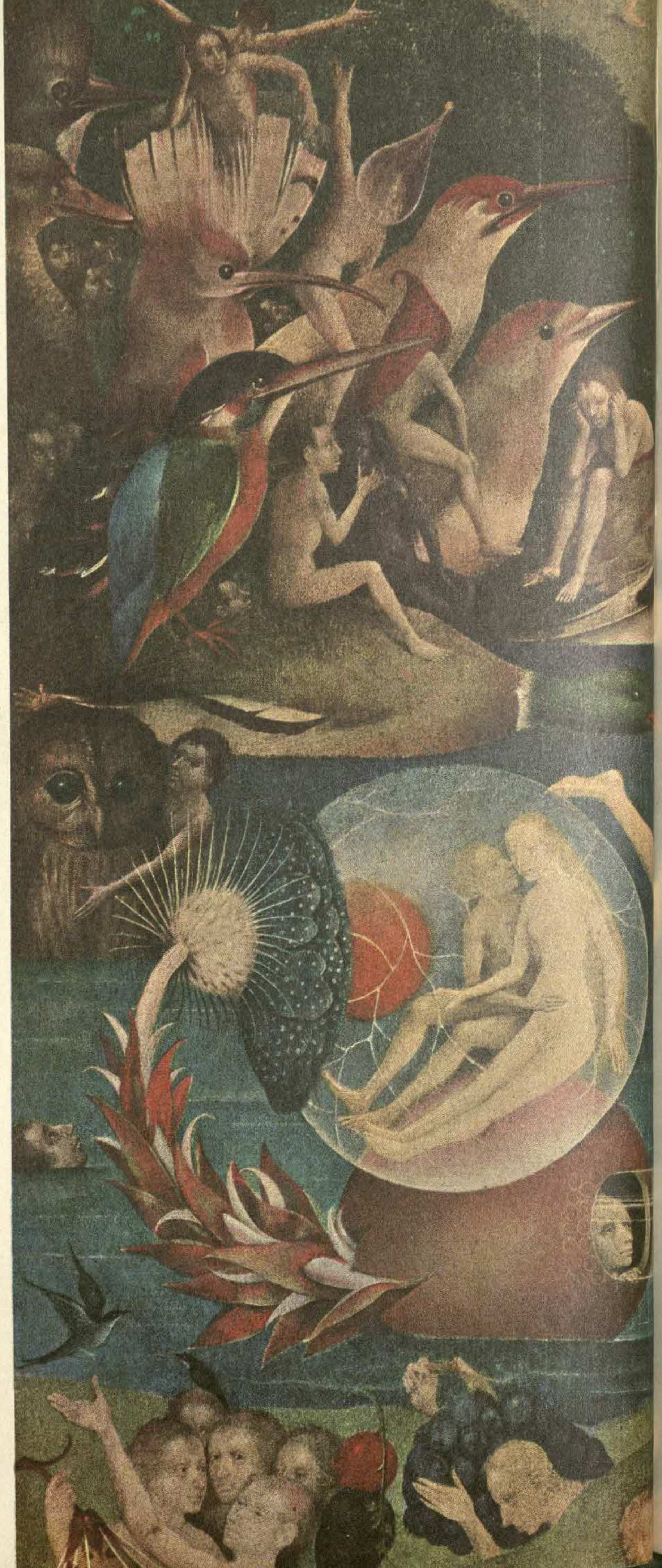
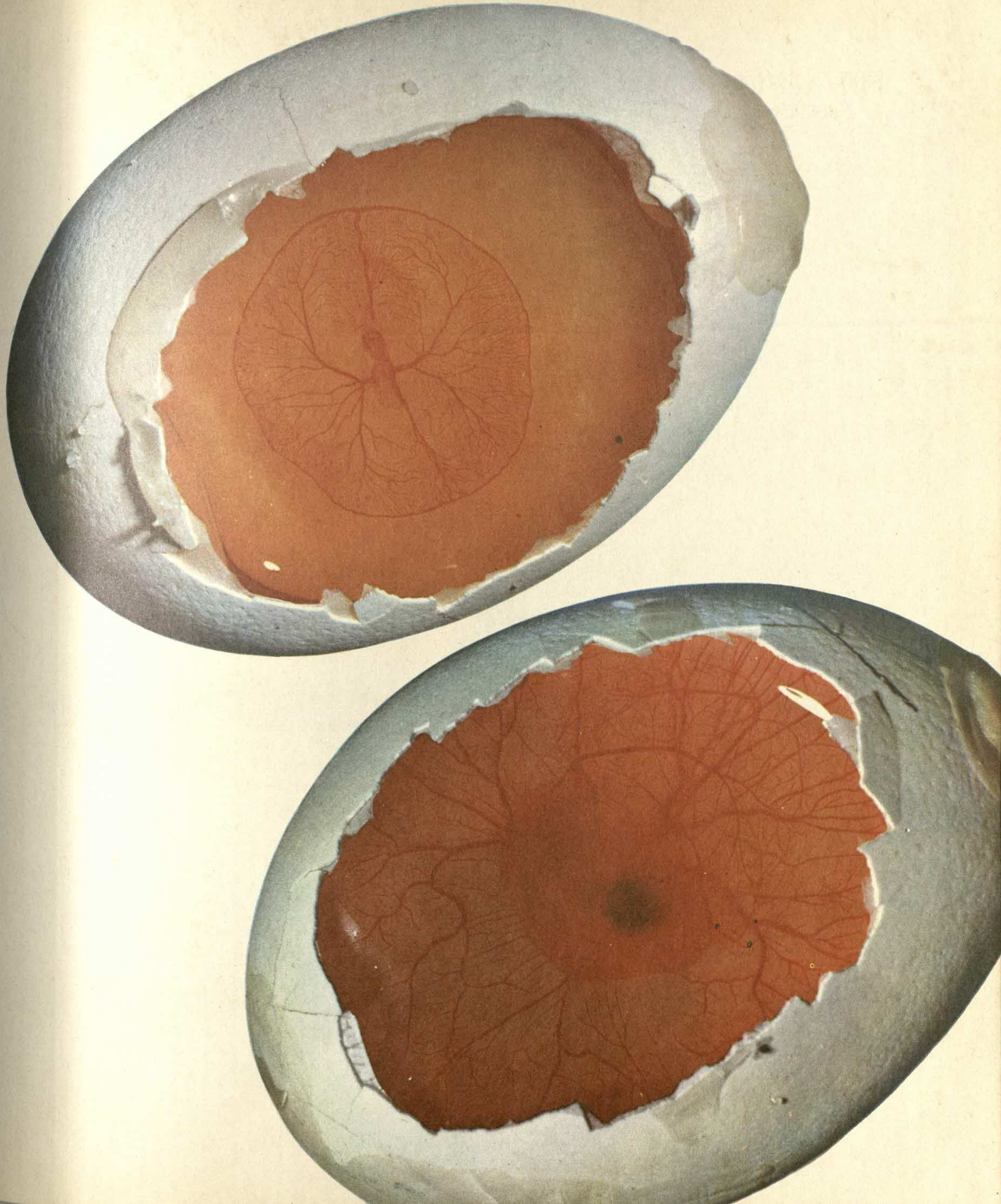
What is life? We know today that it is an uninterrupted chain of chemical reactions, mysteriously begun from the start of the mineral world and dependent on water. From the beginning of time the question has fascinated man. It has inspired, we may suppose, this strange composition of Hieronymus Bosch. Does not the profusion of extraordinary plants and bizarre creatures of the sea symbolise the birth of the world and the diversification of species? Do not the man and woman in the giant bubble suggest the initial great cell of all life's possibilities? Looking at this visionary painting by Bosch we see this initial cell as the biologists see it. It is the egg from which the living organism will emerge.



Right: Part of the 'Garden of Delights' by Hieronymus Bosch, reproduced by permission of Pierre Tisné. Opposite page: Hen's eggs on the third and sixth days of incubation. (Photos Lod).



THE LIVING UNIVERSE
An Encyclopaedia of the Biological Sciences

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The living organism

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Introduction

Max Ferdinand PERUTZ, CBE, FRS

Nobel Laureate, Chemistry

Rutherford is supposed to have said that science can be divided into physics and stamp collecting. By physics he meant the understanding of fundamental mechanisms, while he used the terms 'stamp collecting', somewhat patronizingly, to include observing, describing and classifying, occupations which satisfied many biologists of former days. Modern biology has become more like physics, and its new face is reflected in these volumes.

At first sight they may look as though they are meant for the 'popular science' addict. In fact, this encyclopaedia is a serious work of learning. It is written for the layman, but a basic knowledge of science is an advantage for the understanding of many of the chapters. It should be interesting and useful to older school children, to students in the biological and medical sciences and to scientists trained in disciplines other than biology. Gardeners, too, might learn something about the way plants work and amateur zoologists might find their interest aroused by the volume on microbes.

Each volume is written by a panel of international experts, and consists of a collection of separate articles on various aspects, all trying to present up-to-date knowledge as clearly and simply as the subject allows.

You cannot use these books to look up the difference between a stoat and a weasel, or to discover the classification of spiders. This is not an encyclopaedia of Natural History. If you want to know whether the bone you have broken is called the tibia or the fibula, you will search in vain. It is not an Anatomy either. It tries to convey to the reader what plants and animals are made of, and how they develop and work, rather than what they and what their different parts are called. Technical terms are explained in a marginal glossary. Throughout, the emphasis is on the experimental approach rather than on pure observations; in fact, it might have been more appropriate to call this an *encyclopaedia of experimental biology. Finally, man is treated as just one of the manifestations of life, and medical science as part of biology as a whole.*

What justification is there for this unified approach? Surely a butterfly and a cabbage have little in common, except that the larvae of the one might feed on the leaves of the other? In fact, the outward diversity of life conceals an underlying unity in the molecules of which it is composed and of the fundamental mechanisms which drive it.

All living things, including bacteria and viruses, have nuclei in which the hereditary material is laid down in the form of the same chemical substance: nucleic acid. This contains the information for the growth and development of the organism in the form of a chemical code which is probably the same in all species. What varies is merely the amount of nucleic acid, reflecting the amount of information, contained in the nucleus. If we compare the genetic language to the printed word, we might say that the development of a small virus is laid down in a page and that of a bacterium in a small book, while the information contained in a single mammalian germ cell might be compared to a library of five thousand volumes. Such a fabulous amount can be stored in one microscopically small cell only because the genetic writing is on the atomic scale.

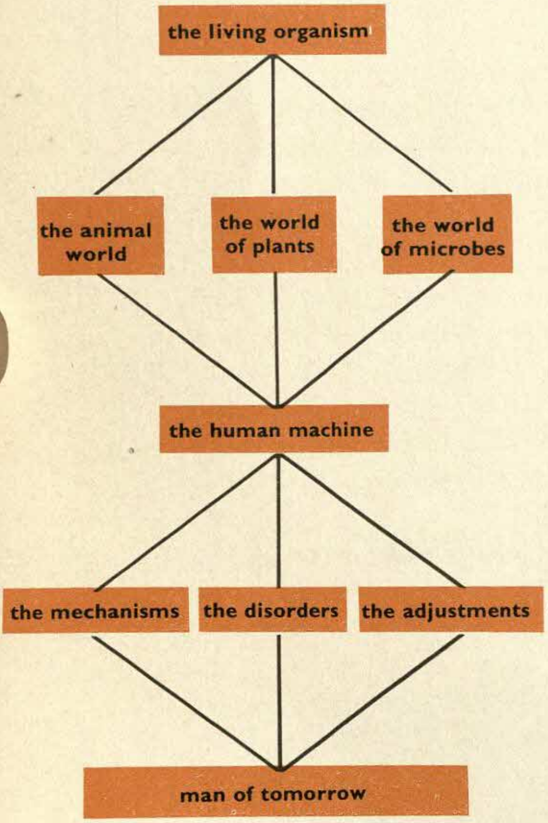
As might be expected, the mechanism by which the genetic information expresses itself also appears to be similar in all forms of life. Probably each paragraph, or gene as it is called, of the genetic script determines the synthesis of a protein, molecule or enzyme, which serves to speed up a specific one of the many different chemical reactions on which the development of the organism depends. One bacterium may be capable of producing several thousand different enzymes. We do not know how many more exist in the tissues of higher animals. The important point is that enzymes catalysing the same chemical reactions in bacteria, in plants and in animals probably have similar atomic structures and do their work in the same way.

There thus exists a unity of life at the molecular level which is essentially a unity of biochemistry. An understanding of fundamental biochemical mechanisms is therefore essential to biology and medicine, and this is why the authors, after devoting much of the introductory volume to biochemistry, again and again return to the biochemical aspects of metabolism and development. But the unity also means that the study of any *form of life is relevant to an understanding of life as a whole, including man. Do not be surprised, therefore, to find as much space devoted to microbes as to plants or to animals, because more fundamental knowledge has been gained in the past twenty-five years from studies of micro-organisms than of all the higher forms of life combined. It is only in the simplest forms of life that basic biological mechanisms can be studied in isolation. Once found, they may provide the clue to the more complex manifestations of the same mechanisms in the higher animals.*

I used to smile at biologists who spend their entire lives studying the differentiation of slime moulds or the behaviour of flatworms, but I have learnt better now. Solutions to the great outstanding problems of medicine may well come, not only through a study of man, but also through ingenious experimentation on some lowly species. This encyclopaedia introduces the reader to some of the exciting discoveries made in studies of viruses and unicellular organisms.

Books of this kind are often criticized for falling between two stools, being too technical for the layman and too shallow for the expert, but I think we should be thankful for this brave attempt to draw together and present as one whole the tremendous output of specialized knowledge in the biological fields. In biochemistry alone, two hundred pages describing new research are being published in scientific journals every day of the year. To do justice to what is known in biology requires a large library, and no encyclopaedia could possibly hope to do so, but these volumes try to give the reader some idea of established knowledge and of the directions in which new knowledge is being sought.

PLAN OF THE ENCYCLOPAEDIA OF THE BIOLOGICAL SCIENCES



The basic problems of biology

Etienne WOLFF

> *Only by reproducing natural conditions of existence can we have some influence on the forces of nature, and we can act more easily on these conditions after they have been analysed in detail and reduced to the greatest simplicity possible.*
>
> CLAUDE BERNARD

How often have we heard it said that the 'secrets of life' are still undiscovered? Yet there is no more a secret of life than there is a secret of matter or of mind; for everything is mysterious until it can be explained. Of course, there is no doubt that some sciences are more advanced than others, so far as explanations are concerned. Thus physics is more advanced than biology, because the phenomena studied by physicists are relatively simple and can often be expressed in mathematical laws. The living world is infinitely more varied and more complex than inert matter. But the concept of matter which forms the back-cloth of physics is no less problematical than the concept of life itself and the final explanation of such phenomena is not likely to be discovered in the foreseeable future. Will it ever be? Will reality ever be expressed in a series of equations, such as the famous 'universal equation' sought for by physicists and mathematicians? Can it be enclosed in an abstract idea, from which it will be possible, to deduce, as Spinoza tried to do, all material, living, and psychic reality?

Does life lend itself to such abstract modes of expression? Would it not thereby lose its content, its essential features, its richness and its spontaneity? We cannot answer this in the present state of science. Nor can we answer the question: will it one day be possible to create life? This question permeates in fact all scientific and secular enquiries.

Before tackling these problems, scientific explanations endeavour to classify these phenomena, to study the general properties of 'facts', to establish laws. There are many ways of approaching the problems concerning life. The dispersal of research amongst so many autonomous specialities proves this. But doesn't life lose its unity when it is thus cut up into sections? The study of living matter is nearly always carried out on a substance from which life has already been removed. A chemical analysis of cellular components requires that the organism itself be reduced to a corpse and the substances of life irrevocably broken down.

Mitosis of a garlic root: the study of living matter applies to a substance from which life has been removed.

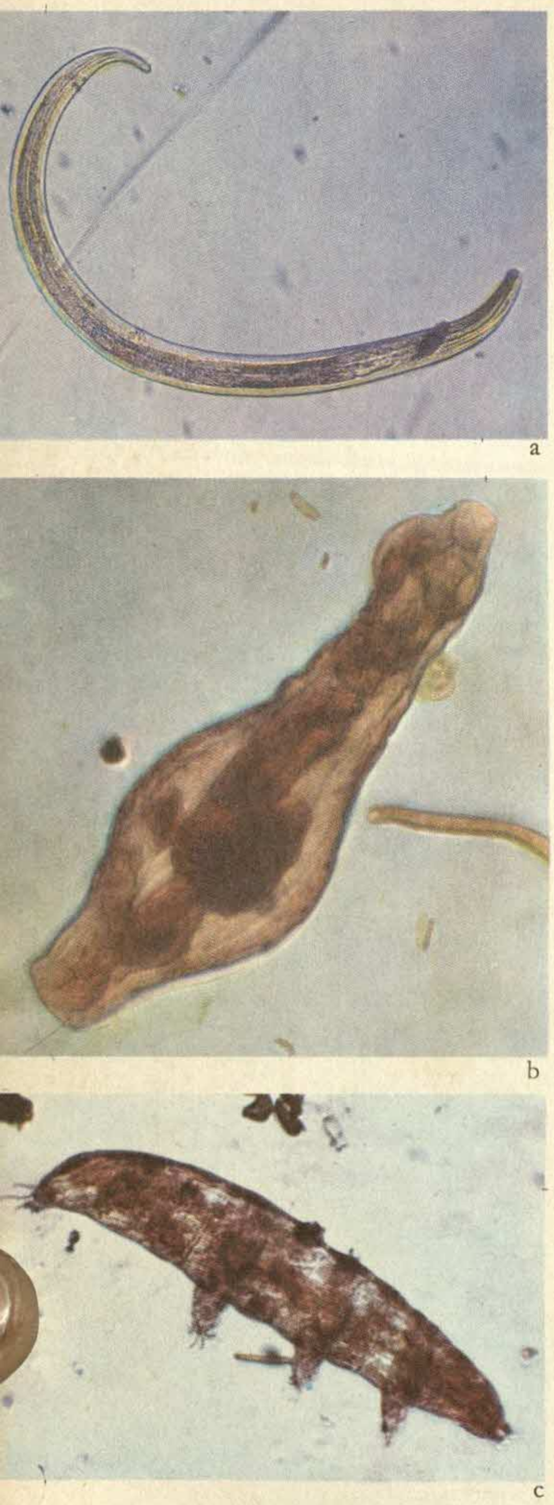


Fig. 1. These minute organisms, a tenth of a millimetre in length, have powers of resistance which much larger beings might envy. Dehydrated for months, preserved at very low temperatures, Nematodes (a), Rotifera (b) and Tardigrada (water bears) (c) are revived by a single drop of rain falling upon them (photo Lod).

A morphological study of cellular structures is generally carried out on fixed, that is devitalised, substances, where an attempt is made to maintain the living appearance. Some physiological researches analyse exchanges between the environment and the organism; they study the transformations of organic substances, establishing that which enters and that which leaves the organism. But sometimes the organism itself is almost excluded from this study. In all branches of biology innumerable examples could be given of this sub-division of interests. Such sub-divisions are in fact necessary because, in order to carry out an analysis, the difficulties must be spread out.

But the specialist, as well as the reader wishing to learn, should not lose sight of the unity of life behind all these varying aspects, nor the vast problems which arise. These cannot be tackled directly but must be got round by some détour. Science proceeds in this way, seeking out the weak spots of an apparently impregnable fortress. Through continuous study and research on limited problems, one tends to forget that the larger problems are themselves real and of a disturbing, even dramatic, actuality. They have indeed a living solution, apart from dried-up theories set out in books where they lose their reality. The problem of the origin of life has a solution, but we do not know it; the mechanism of evolution has a solution and we hesitate between various contradictory theories.

The man of science seeking to raise the level of his research must always bear in mind the greater problems when examining the smaller ones; he must not remain restricted by his speciality. Such is the aim of this book.

Life's power to expand

Let us therefore consider the world as a whole, from some distant vantage point, as would an observer on another planet. We would first of all be struck by the fact that life covers the Earth, as does the possible plant life on the planet Mars, which observers have recently described as changing colour from season to season. Life has spread everywhere, or almost everywhere, except in deserts, on high mountains, in the extreme polar regions and in the depths of the oceans. Nothing can resist life which adapts, models, and takes over all available space. Plant life covers almost the entire surface of the Earth, animals abound, and both jostle together in the higher layers of the oceans. Throughout past millenia, they have not only colonised the surface of the globe but have also modified it greatly. Much sedimentation consists of animal and vegetable remains, pressed down into mile-thick layers in millions of years. Where crystalline rocks emerge, issuing from some molten magma, it is not certain that they belong to the original crust of the earth; for many of these rocks, such as gneiss and granite, are ancient sedimentary formations which have been melted. In those regions where the earth seems to have opened up to reveal the sequence of the ages, such as the Grand Canyon in Colorado, for example (fig. 2), one might imagine that from this calm and mysterious grandeur of deposits undisturbed by time the bottom of the valleys might reveal the original rock, earth from the beginning of the world before life began. But this is not so. Beneath the piles of horizontal sediment, in the very depths of the ravines, we find the remains of a mountain chain, with its warpings, its intertwined strata, showing, in spite of its metamorphoses, that life inhabited them long ago.

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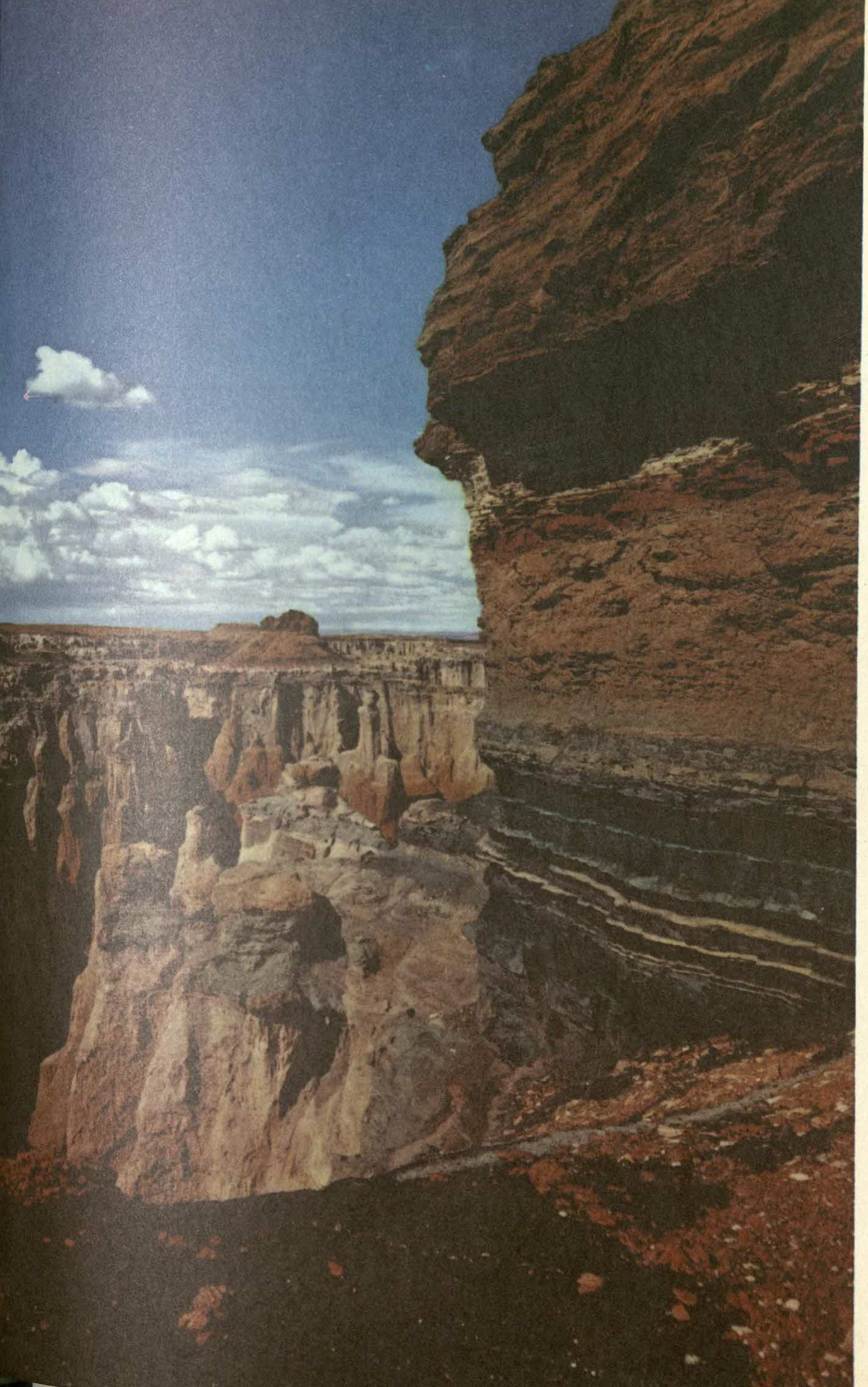
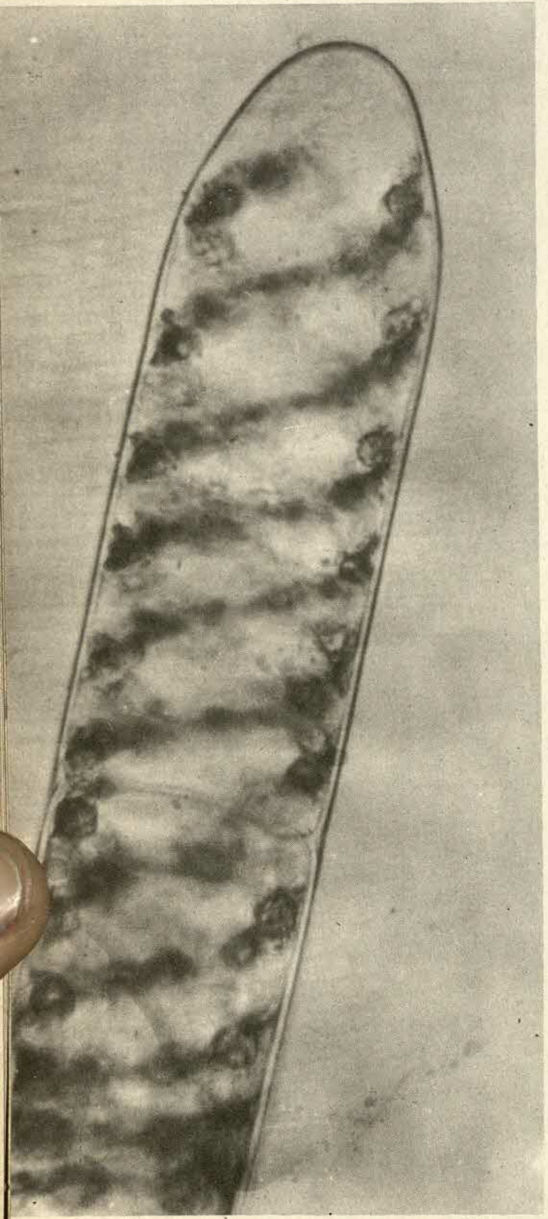


Fig. 2. To descend into the depths of geological strata, to survey the layers belonging to past eras is to retrace time. The Grand Canyon in Colorado, a unique site, shows us the layers of geological strata which make up the history of the earth's crust. The more recent layers cover the more ancient. The history of life can be read from bottom to top (Abbot, Rapho photo).

These considerations show that life's power to expand is unlimited. All that is required is merely a little water, a little air, a little light, a few mineral salts and there emerges a rich vegetation which will soon be covered by a voracious fauna. Space is seldom left empty. Think of land which has been swept by fire, destroyed by some catastrophe: an explosion, a volcanic eruption. A few months, a few years at most, and no trace of the disaster remains. Life has wiped it out.



Spirogyra *(a microscopic green alga): an artificial pond or an abandoned tank will first be colonised by a population of bacteria, amoebae, infusoria, and microscopic algae.*

And on this globe invested with life, man has come to create order, to put his mark on life. He imposes his discipline on living beings, on inanimate bodies. He alters and falsifies the scenery. It is not necessary to recall how crops, forests, towns, roads and dams change the face of the world, which only remains true to itself in a few protected spots. Human nature adds to the transformations of the physical world which nature, living but blind, has inflicted on it. The two orders are complementary or in opposition, increasing still further life's hold over matter.

The fragility and resistance of living forms

Living beings, in spite of their fragility, have powerful means of defence and reproduction. Their resistance, their means of multiplying, are of an almost infinite variety and richness. Many living creatures succeed in being at one and the same time very vulnerable in the ordinary conditions of life yet amazingly resistant to the roughest handling. Cells, organisms which, in their vegetative state, cannot withstand even slight variations in temperature, undue dryness, slight atmospheric or nutritive differences, have resistant forms (spores, cysts, etc.) which tolerate in an incredible way the harshest treatment. Tiny worms, such as the Nematodes, Rotifera, small Tardigrada (fig. 1, a, b, c) can survive for years, in the adult state, in a dehydrated form, at temperatures near absolute zero. Fresh tissues can, when working normally and under certain conditions, be frozen rapidly to temperatures around – 196° C and recover their vitality when reheated.

Organisms with resistant forms are the first to colonise virgin ground. An artificial pond, an abandoned tank, will soon be inhabited by bacteria, infusoria, amoebae, microscopic algae. These are the pioneers of a vast colony, soon to become the victims of more highly organised species which fate will inevitably and by the most devious means bring to these places.

Living matter is extremely fragile and extremely enduring; such are its inherent and contradictory characteristics. Mass destructions are always compensated by the surge upwards of new arrivals. Thus is the surface of the Earth invaded by life, which breaks down and transforms inert matter to make a new substance, infinitely more complex. We must always remember that organic compounds derived from inorganic were first made by organisms long before they were created by those other living beings, chemists.

It has been said that 'Life is a disease of matter'. This is, of course, a statement more flippant than scientific. But it stresses the idea that inorganic matter is undermined and eaten away by living beings which thrive on it like vermin and transform it radically.

Life's limitations

When embarking on a study of life, one should endeavour to define the characteristics

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of living things, but this attempt would only develop into a mere school-book essay, and, in any case, the reader will be able to find within this work concrete answers to the question; for the moment we will merely define life's domain. With the discoveries of the last decades, the frontiers between living and inanimate matter seem less clearly defined. No doubt the basic criterion of organisation, more particularly cellular organisation, can be applied to most living beings, especially since the recent discovery that bacteria possess a nucleus like other cells. But filterable viruses and bacteriophage, now visible under the electron microscope, whereas previously their very existence was only known by deduction, evade such a criterion; their organisation is molecular, that is to say, chemical. Some, such as the tobacco or tomato mosaic virus can exist in crystalline form, as Stanley showed for the first time in his sensational discovery (1935). Yet when placed in a solution, they can reinfect organisms and propagate themselves (fig. 3). These crystals and molecules are very complex; they are large and of high molecular weight compared with the molecules usually found in mineral or organic chemistry. But does the complexity of a molecule suffice to define life?

Some biologists and chemists tend to deny the living nature of these curious 'beings'; they are biological catalysts possessing few or none of the enzymes found in living cell as essential agents of any breakdown or synthesis. They do without enzymes, employing those substances which are formed by the cells they feed on. But they retain two fundamental characteristics shared by all other living matter: they are mainly composed of nucleoproteins, complex molecules found in the nuclei of all cells of animals and plants which determine hereditary characters, and they are also capable of multiplying, being thus endowed with those self-reproducing properties which are perhaps the most fundamental signs of life. Are these beings, deprived of cellular organisation and without enzymes, the simplest form of life? Is it with these that life first made its appearance on the Earth? To this the answer must be no, and we shall shortly see why.

At the other end of the scale in the living world we find beings endowed with consciences, who know how to wait, how to choose, how to foresee, and how to solve a problem. At what level does the spiritual and psychological life begin? In fact, there is no barrier between life and spirit, between vegetative and psychic life. Higher animals and man have higher faculties which give more power and more freedom, but every organism has a psychic life, no matter how elementary. The protozoa and bacteria, have impulses directing them to their goal and defensive reactions against external influences. These reactions, although predictable, are none the less to some extent spontaneous, presenting a personal coefficient which varies with the individual: the reactions of populations can be predicted, but not the detailed behaviour of a given individual. These reactions, or tropisms, become refined as the organs specialise in receiving stimuli. *Euglena*, a flagellated Protista (fig. 4) possesses a small sensory organ, a coloured spot which perceives luminous waves and enables it to direct itself in regard to light. There is a great gap between this elementary organ and the perfected eye of an octopus or those of the vertebrates, yet the main characteristics of the organ of sight are already present in this rudimentary sensory apparatus.

Psychic life begins with the irritability which is inherent in living matter. Animal psychology must seek to detect and study all levels of behaviour: tropism, instinct, and intelligence, but to define the boundaries is difficult, as these forms of psychic reactions coincide even in complex living beings. It will be noted that psychic life has mainly developed in the animal kingdom, remaining very elementary in plants, especially higher plant life. This is because most animals must move

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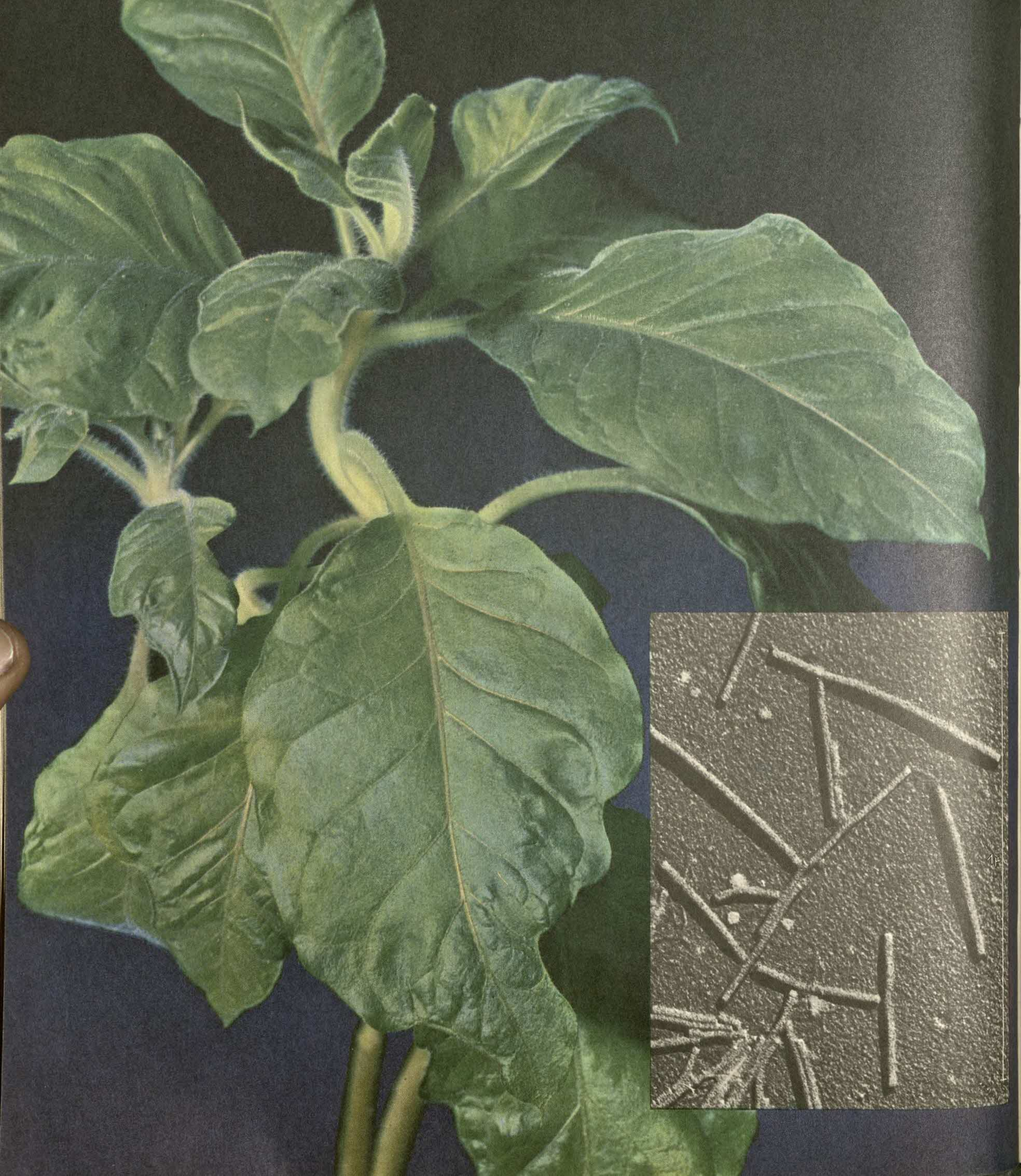
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Fig. 3 (p. 16). Virus of the tobacco mosaic disease: structure seen under an electronic microscope, showing its effects on a tobacco leaf (Werkfoto Carl Zeiss and photo Lod).

in order to obtain their food and to be able to reproduce themselves. They must make an effort, take the initiative. When certain groups or species solve this problem by adopting the sedentary life of fixed forms or parasites, the sensory organs very often atrophy or disappear, as do the organs used for movement. The adult parasite often differs completely from the forms of the same group which continue to lead a free life.

In a grandiose picture, the French philosopher Henri Bergson contrasted the lethargy of the vegetable kingdom with the turbulence of the animal: the former asleep for ever in safety and immobility, because it has found the ideal solution to the problem of feeding which does away with all effort, the other always on the move, living in a state of worry and anxiety, always seeking new means of fighting for existence: it gains in freedom, initiative and power, sometimes even succeeding in breaking away from the servitude of matter. So life becomes conscious of itself and, as a result, becomes capable of acting on its own destiny.

Cycles of organic life

A very remarkable aspect of living nature, considered as a whole, is the fact that, in the chaos of beings jostling each other to obtain territory and acquire food, there is a balance, a spontaneous order which extends beyond individual life and death; these are the cycles of organic matter whereby all beings depend one on another and whereby matter, after many transformations, returns to its initial state. The most interesting cycles are those of carbon and nitrogen.

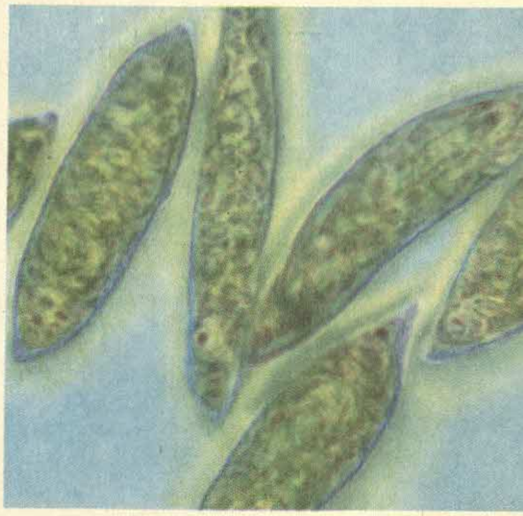

Fig. 4. Although it belongs to the unicellular group, Euglena *—an alga, part animal, part vegetable—has a sensory organ, enabling it to move towards the light. This is a sort of primitive visual organ (photo Lod).*

Green plants play a vital part; for without them animals would have neither oxygen nor food that they could assimilate. Plants transform mineral substances into organic ones; hence they are described as 'autotrophes'. They extract carbon from the carbon dioxide of the air, take the nitrogen required to synthesize proteins from the nitrates in the ground, and then return to the atmosphere the oxygen required for the breathing of all living beings. Green plants only need mineral substances, whilst animals, whether herbivorous, carnivorous or omnivorous, are invariably directly or indirectly dependent on plants. After death, animals restore to the soil the broken-down organic substances which bacteria retransform into nitrates to be assimilated by other plants. This difference in nutritional habits is the essential difference between animals and plants; it can even be found in the needs of animal and vegetable cells isolated in a culture, where the former are much more demanding that the latter. By eating each other, by attacking dead material and excrement, many organisms collaborate to maintain life on earth.

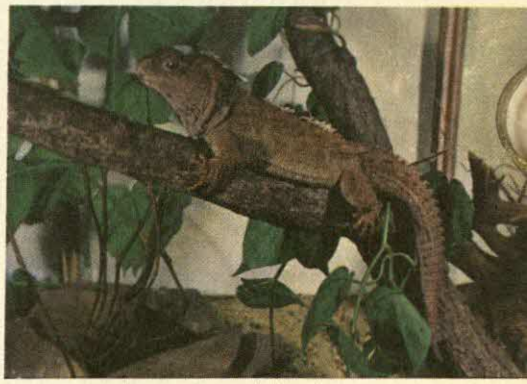

Fig. 5. Reptiles multiplied greatly during the Secondary era and we are amazed by the variety and significance of the fossils found. One of these groups, the Rhynchocephalia, still has a living member in New Zealand, the tuatara. Its preservation, like that of other 'living fossils', due to geographical isolation, makes it a precious proof of past ages for the biologist (Senckenbergisches Museum, Frankfurt).

If certain species have an assigned place in these fundamental cycles, it is not because of

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any pre-established harmony but rather because of the competition for available space. Living beings have adapted themselves to the most varied physiological conditions. No doubt the most adaptable are the bacteria which have many different ways of feeding. These cover the entire range: some live on other organisms as parasites, for example, the pathogenic bacteria, carriers of disease, or in symbiosis, such as the harmless and even useful bacteria in the digestive tract which are essential for certain fermentations; others attack decomposing organic matters; others again are autotrophic, like sulphur and soil bacteria.

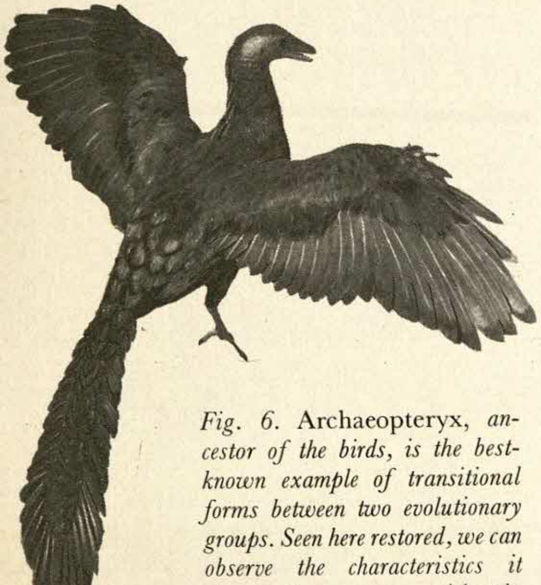

Fig. 6. Archaeopteryx, *ancestor of the birds, is the best-known example of transitional forms between two evolutionary groups. Seen here restored, we can observe the characteristics it shares in common with the reptiles from which it has descended and the birds which it presages (photo British Museum, London).*

The reality of evolution

Biologists are almost unanimous today in admitting the fact of evolution. Palaeontological information does still leave vast gaps between certain phyla; we do not really know of any intermediate form between the vertebrates and invertebrates, nor do we know of any between most of the zoological phyla. *Archaeopteryx* is a bird with archaic characters which bring it closer to a reptile than to a present-day bird (fig. 6). But there are obvious palaeontological series, such as we have already mentioned; the succession of fauna, the order of appearance of the groups, everything favours evolution. There could be no better example than the vertebrates. The order of appearance of the various classes coincides with the hierarchy of complexity recognised in our zoological classifications. It is remarkable, too, that early forms, today mostly extinct, did not greatly resemble those we consider to be typical nowadays of fish, amphibia, reptiles or mammals. There are, however, 'prophetic' forms presaging not only the group to which they belong but also future groups. Thus the Stegocephalia, amphibia of the Devonian period, were the forebears not only of other amphibia, but also of reptiles. Reptiles of the Palaeozoic and early Triassic period were the direct ancestors of mammals which they presage so clearly that it is difficult to place some of them in one category rather than in another.

Crossopterygian Fish of the Palaeozoic, of which the coelacanth is a miraculously preserved example, are probably the common ancestors of Dipnoi-fish and amphibia: their paired fins, upheld by bone-like structures, announce the limbs of Tetrapod or four-legged vertebrates (fig. 7).

And how many other proofs can be found of the reality of evolution, not only in fossils but in present-day creatures? True, these are the product of a long history, the ramifications of a tree from which the trunk and main branches have long vanished. Yet some of the primitive forms have continued to our own times. We have numerous survivals of past ages, living fossils such as *Lingula*, belonging to the brachiopods; the nautilus, belonging to an extinct family of Cephalopods; the coelacanth, already mentioned, and those anachronous reptiles of another ancient group, the tuatara, which have been preserved, like other living fossils, owing to geographical isolation (fig. 5).

Even when the primitive species has not descended to us intact, many of the present forms show themselves to be without any doubt near relatives of the unknown ancestor. *Amphioxus* is not the direct forebear of vertebrates, but how nearly so! A certain specialised function of the gills shows that it drifted from

* * *

the direct line but its whole body announces the vertebrates, just as does that strange group of tunicata, which seems to have returned towards the invertebrates.

It is unnecessary to study all these primitive or prophetic creatures to see before us the general plan of evolution. It is a commonplace to say that the history of the individual retraces that of the species, that ontogeny reproduces phylogeny, although thus expressed this is not in fact true.

But it is undeniable that the embryos of all vertebrates have, at their inception, the same organisation, so that it is sometimes difficult for a practised observer to distinguish one from the other. All have visceral and branchial arches (but not all have gills), all have a heart, at first tubular and not divided, all begin with the same uro-genital system and the brain is always formed from the same vesicles (fig. 11).

These ideas have often been expressed theoretically and diagrammatically, sometimes losing sight of the concrete reality. But the embryologist never ceases to be astonished by these similarities: he can watch the formation, as real organs, of three succeeding types of kidney in the higher vertebrates, whereas the lower creatures develop only two. The first of these organs is usually only a vestige, repeated 'for memory's sake', but the second is functional and is the embryonic kidney for those species which acquire a third one.

And how revealing are these transient devices, those rudimentary organs to be found in any representative of a species. Sometimes they disappear entirely, sometimes they leave some vestigial traces. They play no part in adult life, sometimes none even in the embryo. Yet there they are, as if to revert back to the primitive plan, to remain faithful to the past. It is truly astonishing to observe all vertebrate embryos with two sets of sexual organs, male and female, as if the choice were to be left to the organism, whereas, in fact, except when there is some malformation, sex is fixed at the moment of conception. One of the sets almost disappears, leaving a mere vestigial relic in the adult, the possible cause at a later date of a cyst or tumour. Does this presence of bisexual characteristics revert to a distant past? A final example: a relationship has been suspected between worms and molluscs, although they differ greatly in the adult state, yet many aquatic invertebrates of both groups have, at birth, exactly the same ciliated larva, the trochophore, with the same organs, and identical appearance. However, the trochophore is to be found in so many varied groups that it cannot be due to mere resemblance or to what biologists would describe as convergence due to a similar mode of living.

The origin of life

For logical reasons, it is generally believed that all animal species have a common origin. This theory has not been proved, as there are important gaps between certain phyla. It is not known whether life appeared on Earth once or many times. There is a monophyletic theory (a single genealogical tree) and a polyphyletic one (several genealogical trees). Since we must presume that the first living creatures were very simple in form, unicellular or even acellular, the polyphyletic hypothesis explains no better than the other the origin of multicellular organisms. Almost

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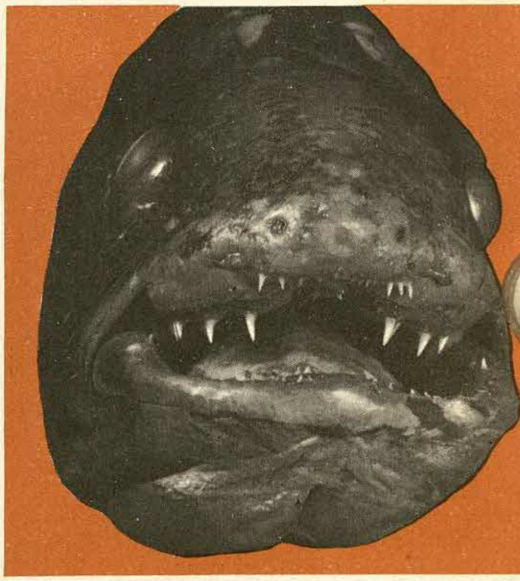

Fig. 7. Some species have been preserved thanks to diverse factors in their original state, hence their great interest for biologists: such is the Coelacanth, a member of a group more than 300 million years old, which seems to defy extinction: the recent discovery of live specimens has enabled us to peer into the distant past (Le Cuziat, Atlas-Photo).

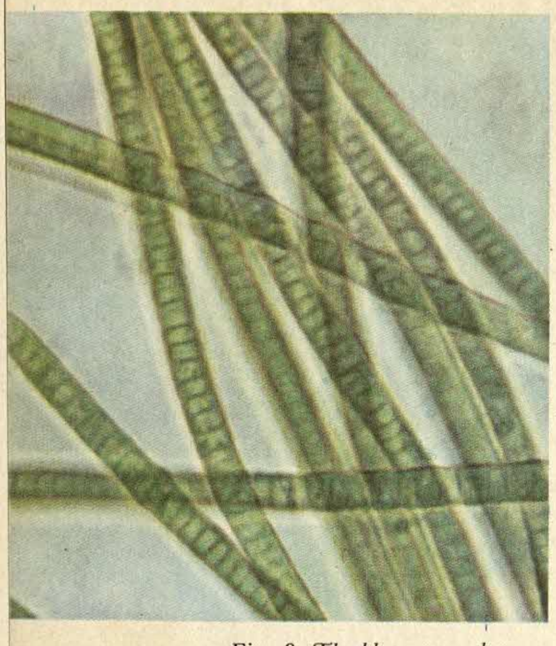

Fig. 8. The blue-green algae or Cyanophycea, which, by reason of their structure, are closely akin to bacteria, are autotrophic; that is to say, they feed solely on mineral substances as all the first living beings on the Earth must have done (photo Lod).

Fig. 9. Can an organ as complex as the eye of a cephalopod arise merely as the result of evolution? Or is the potentiality for such perfection present within the organism from the origin of the species? (photo Lod).

all living beings, it must be remembered, go through a unicellular stage at the moment of sexual reproduction. Male gametes or spermatozoa, in animals or plants, resemble protozoa of the Flagellate group, and the female gametes of many algae preserve this characteristic. So it could well be that multicellular organisms are derived from ancestors belonging to Flagellates (fig. 10).

How did life first appear on the Earth? Palaeontology reveals nothing concerning either the origin or the appearance of the first living beings. No matter how far back one goes in the geological record, sedimentation shows invertebrates and plants at a quite advanced level. Palaeontology could not in fact reveal the conditions under which life was created and other bold and ingenious hypotheses have been evolved.

How were the first living creatures constituted? Were they autotrophic, like green plants, or like Protista, supplied with chlorophyll? Or did they, like the blue-green algae (Cyanophyceae) (fig. 8) closely resemble in structure the bacteria? But these creatures already have a complex organisation and their chemical constituents are highly evolved. It is difficult to imagine that such a complex molecule as that of chlorophyll could have been synthesized spontaneously. One cannot imagine either that filterable viruses are the most primitive organisms; rather are they the lowest: for, although they have no cellular organisation, 'living' in a molecular state, yet they require other living cells in which to live and, unlike many pathogenic bacteria, they cannot be cultivated apart from living matter. They are therefore parasites and their very existence presupposes the existence of organised animal or plant life.

We are entitled to wonder, however, whether life did not first appear as matter unorganised into cells but having the power of synthesis or building-up, which viruses do not possess. A. Dauvillier has put forward a very attractive theory, based on astronomical and physico-chemical facts, showing how the atmosphere, originally without oxygen or ozone, was ultimately penetrated by ultraviolet radiation of short wave-length and high energy. S. L. Miller's experiments have shown that radiation of this type can lead to the formation of complex organic substances from a combination of water vapour, methane, ammonia and hydrogen under experimental conditions in the laboratory. These components, which must have existed in the primitive atmosphere, would suffice to build up glucosides and amino-acids. The production of asymetrical macromolecules, characteristic of living beings, could be explained by the action of light on the first organic substances formed. Thus organic compounds would have been present on the Earth before life proper and would have reached a high degree of complexity so that the first living creatures could feed on organic substances already present.

The appearance of life coincides with the time when macromolecules came to be endowed with the possibility of reproducing themselves. However, the change from acellular beings to cellular organisms is still difficult to conceive.

From this interesting theory let us remember that it explains more readily the genesis of organic matter than that of living matter. But this is a first step towards finding the key.

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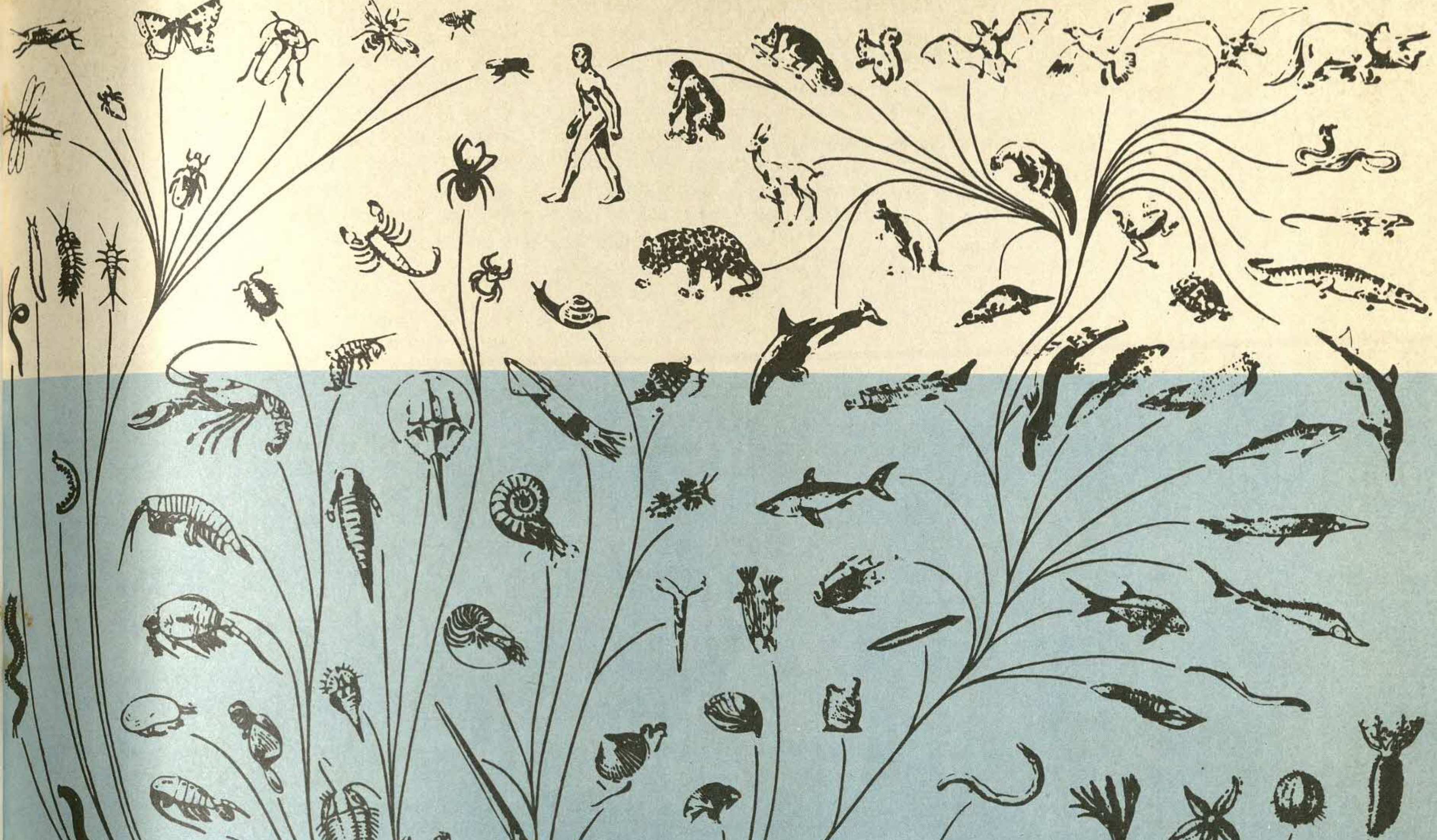

Fig. 10. Evolution is generally accepted nowadays as true. This genealogical tree of the animal kingdom is based on palaeontological evidence and the postulated derivation from older groups of species existing today.

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Spontaneous generation and the creation of life

The problem regarding the origin of life is closely linked to two other problems. The first is that of spontaneous generation, the second concerns the creation of living matter. Can life appear in nature, spontaneously or experimentally? Can one at least imagine that some day it might be possible to create life?

So far as the first problem is concerned, we know that Pasteur proved conclusively that spontaneous generation does not take place under present conditions and it is curious to note that this theory was refuted at the precise moment when the doctrine of evolution was being put forward by Darwin. It is unfortunate that the idea of spontaneous generation must be given up whereas evolution is recognised as acceptable; for if this were not so, we would see every day, almost before our very eyes, multiple evolution beginning and developing. But the theory of spontaneous generation has come back several times to the attack. Always repulsed, it can now only be considered as feasible for the lowest creatures, such as the viruses or bacteriophages. Can one imagine that man may be able some day to create living matter? His mastery over matter, the advances of science in every field have been so great during the last century that some are convinced that this result will be reached. Why could not the conditions—still unknown—under which life first appeared on earth, not be reproduced? Man has done many other things. He has made chemical compounds, some identical with natural substances, others entirely synthetic, filling up omissions made by nature. He knows the simpler substances of living matter, he knows what the constituents of proteins and ribonucleic acid are, their properties and something of their structure. Amino acids can be man-made, some polypeptides can be synthetised, and this is a first step towards manufacturing more complex proteins. One can predict, without too much risk, that man will carry out ever more complex syntheses, and that one day he will find the way to create proteins and nucleoproteins. Will he then have recreated life?

To answer this question it is sufficient to recall that we can at present extract these complex organic compounds as inanimate substances. Yet if we did succeed in preparing such substances by means of synthesis, they would still not be endowed with life, at least during the first phases of research. All living organisms are rich in proteins, in RNA and, apart from viruses, in those biological catalysts which are enzymes, also proteins.

Could an appropriate mixture of these various components become living matter, with its own structure and self-reproducing properties? Would such a mixture have to be subjected to the original conditions—still unknown—under which organised life first appeared on the Earth? Such a phenomenon is considered as most improbable by some biologists and physicists, who calculate in terms of probabilities. Yet this unlikely eventuality did occur once upon a time spontaneously, by chance, so could it not happen again, this time directed by scientific research? It is impossible at present to give an answer to these questions.

Mutations and natural selection

Let us therefore admit that this most unlikely event, the appearance of life, did

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occur once upon a time. How, from an original, primitive enigmatic being, did life diversify into so many animal and plant groups, both those extinct and those still living, all complex and highly organised? If evolution be a fact, the mechanism of evolution is still subject to controversy. The two classical solutions suggested are well known. Lamarck's theory postulates that an organism, having adapted itself during its lifetime to a new environment, passes on the changes which have been brought about to its descendants. According to Darwin's theory, changes occurring fortuitously can be of advantage to the species. An accumulation of such alterations during generations can bring about evolution. Both Darwin and Lamarck assume the possible hereditary transmission of acquired characters.

We cannot discuss here doctrines which have aroused so much controversy, nor present the various arguments for and against. Let us simply say that no concrete evidence has ever been able to prove that any organism can transmit to its descendants characteristics acquired during its lifetime.

But there is other evidence which makes a positive contribution to the problem of the mechanism of evolution. These are mutations (inceptions of heritable variation, changes in genes) which, unknown in Darwin's day and brought to light by de Vries (1900) are concerned with the germinal cells, and not with the somatic cells which, for biologists, designate the cells which inevitably die. So these variations only show themselves a generation after their actual occurrence, like a photographic plate after the photograph has been taken. Continuous or not, important or insignificant, these variations are transmitted as such to future generations. The problem of inheritance of acquired characters does not arise as the entire germ cell is modified, since the chromosomic structure has been altered.

How do these mutations occur? Can they, alone, bring about evolution? They occur spontaneously and accidentally in bacteria, plants and animals. They can be induced experimentally by the use of certain radiations or chemical substances. But they still appear fortuitously, at random, without any rigid control by the research worker. Also, they are more often detrimental than otherwise to the organism. But let us suppose that some mutations are of benefit to the species. Geneticists, reverting to natural selection, the key to Darwin's theory, consider that individuals having these privileges will be better able to fight for their existence. In the competitive fight between individuals for various vital needs, those not having such advantages are gradually eliminated. So beneficial variations can be progressively accumulated by a lineage and from one accidental mutation to another, the species can be modified and progress made on the path of evolution. That it can in fact bring about a 'microevolution', research has now demonstrated to be certain. But can the accumulation of mutations do more, can it transgress the limitations of the species, the type, the order, the class, or even the phylum? Do mutations suffice to explain large scale evolution? Many biologists believe this to be possible. Mutation and natural selection would in this case be the determining factors in evolution.

Nobody contests today the fact that living matter is plastic, malleable, and changeable. There is no doubt either that, in order to change, the organism must be transformable. But the question remains as to whether the internal or external factors have played the more important part in the evolution of living species.

Mandibles of a Coleoptera: can a series of fortunate accidents create such a perfect organ as the buccal parts of an insect?

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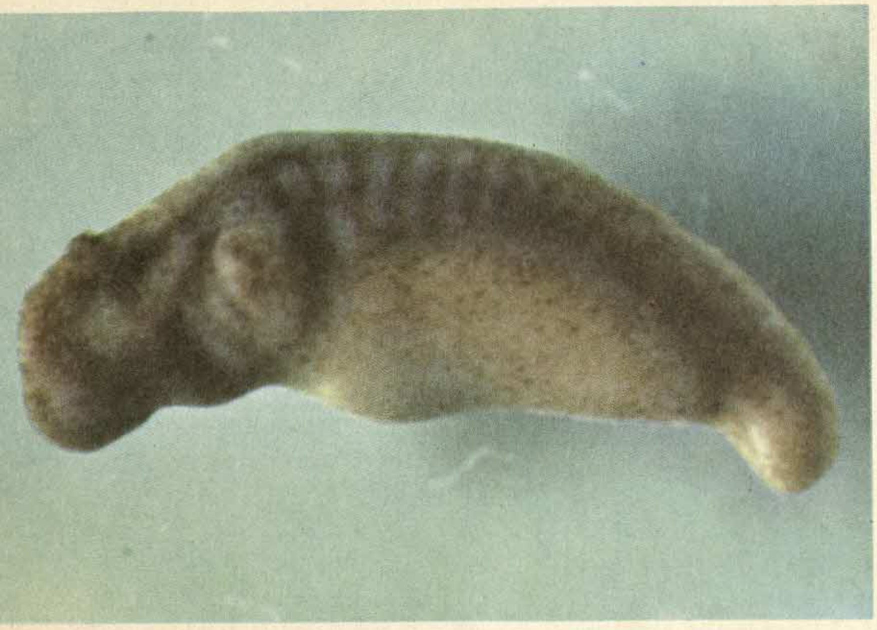
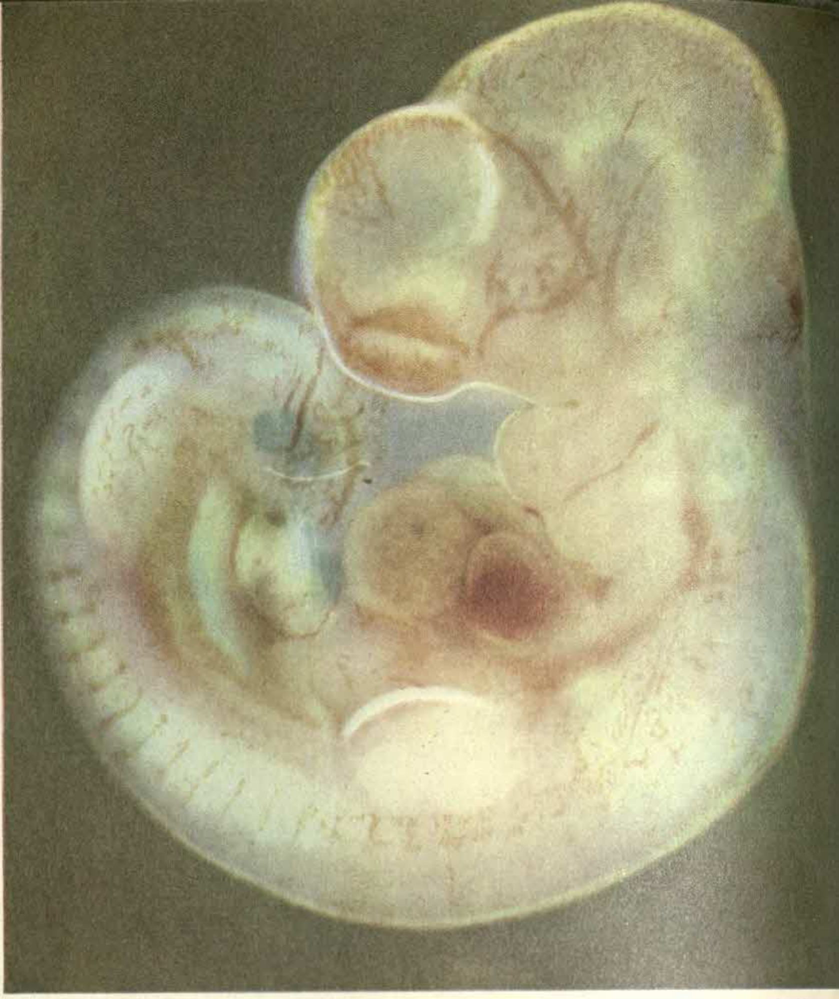
Pleurodela (mag. × 40).





Fish (trout) : tail-bud stage.
Amphibia (newt) : tail-bud stage.
Bird (chicken) : 3rd day of incubation.
Mammal (rat) : 12th day of pregnancy.

Rat (mag. × 30)

Trout (mag. × 25)

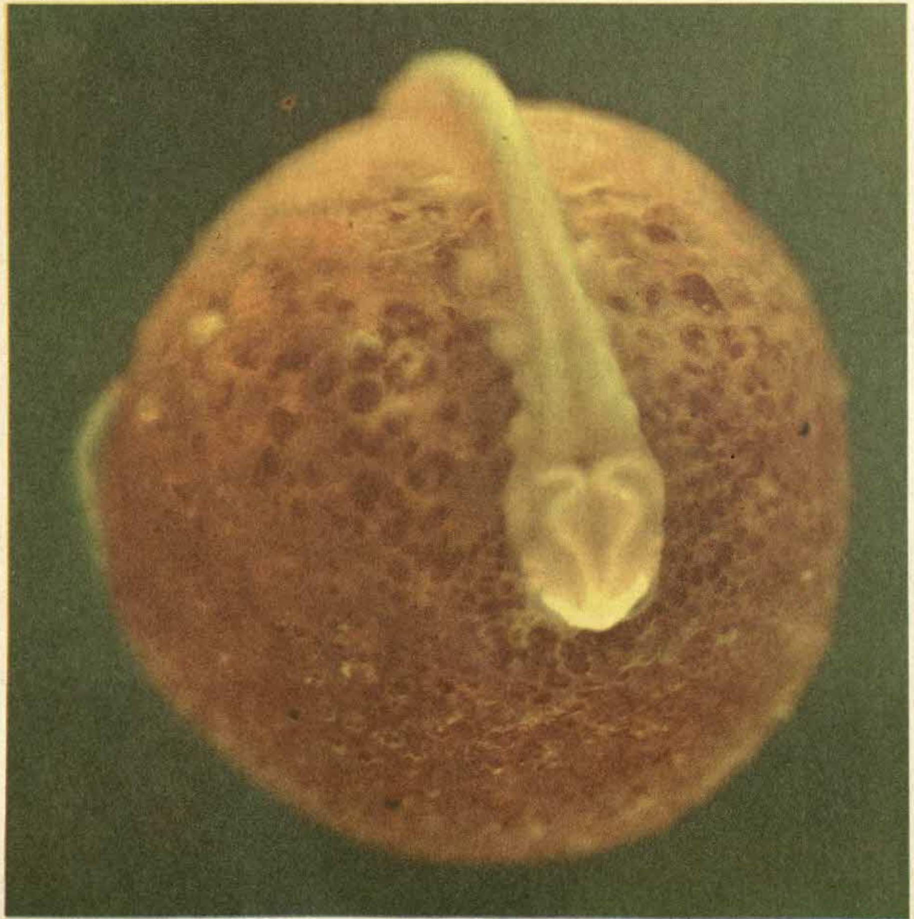
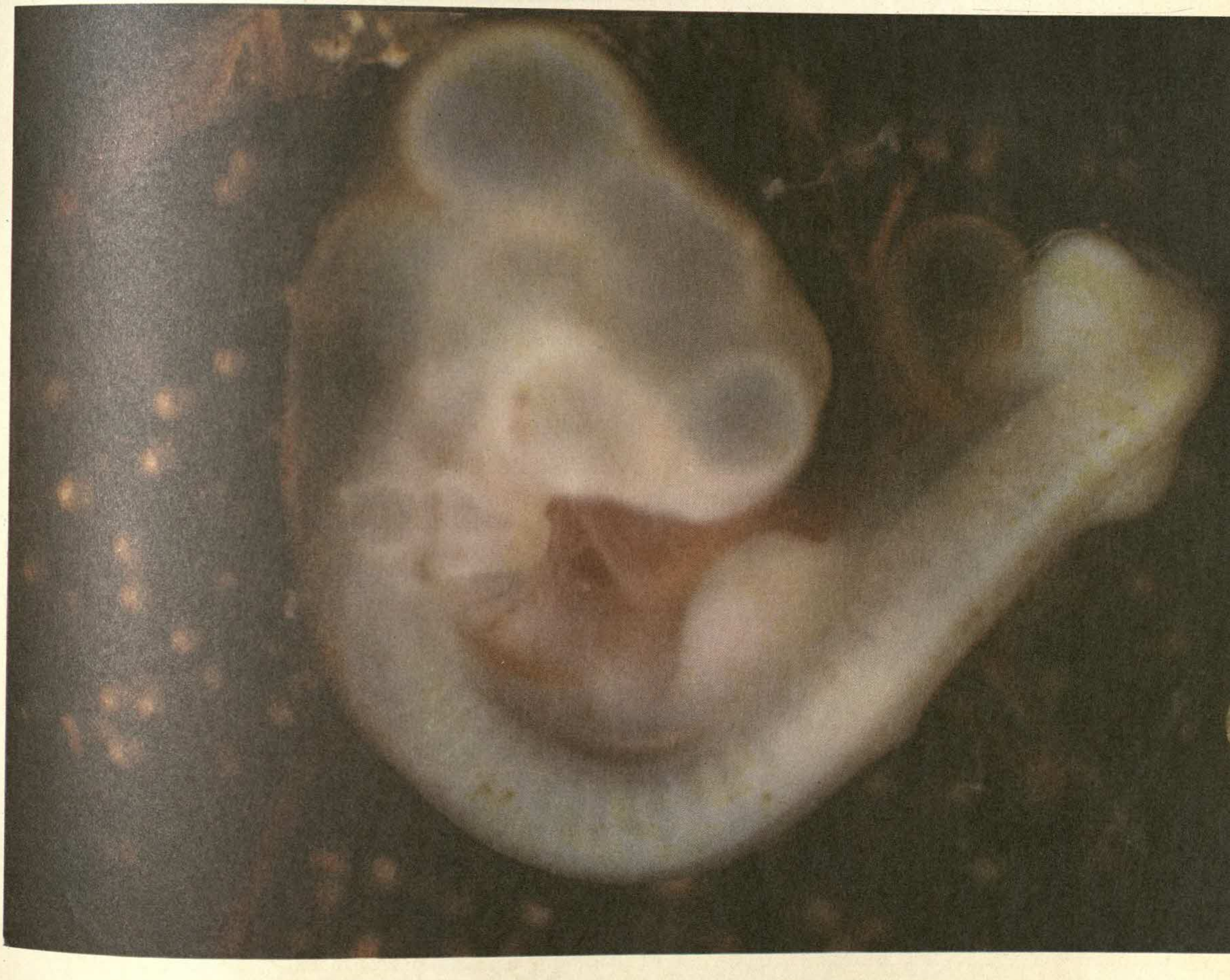


Fig. 11. One of the most convincing arguments in favour of the theory of evolution is the resemblance, at certain stages of development, between species which, later, will differentiate greatly. Thus the embryos of all vertebrates have, at the beginning of their development, the same organisation, so that it is sometimes difficult even for a practised observer to see the difference. All have branchial arches and branchial slits, all have an undivided tubular heart, all have the same urogenital formation. This can be seen on examining the embryos of a fish, a batrachia, a bird and a mammal (photo Lod).

Embryos of four vertebrates

Chick, 3rd day of incubation (mag. × 20).



Development of the individual

Ontogeny, that is the development of the individual, sets more concrete problems which appear easier to solve than those arising out of phylogeny, the evolution of the species. We can see embryonic development taking place before our eyes with the use of our very delicate optical instruments. Detailed experiments have enabled us to analyse certain mechanisms of development, and experimental embryology has gone from one success to another since the end of the nineteenth century.

By diverting development, by setting up obstacles to growth, abnormal beings, monsters which may or may not survive, have been created, giving us information about normal ontogeny. But on occasion even the most traumatic operations do not alter development from its normal path. Results of this sort are almost incredible; for they show the resources of living matter, its plasticity, its ability to regulate itself at a time when future organs are only potentially in existence, when they have yet to be given a specific position in the egg or embryo. The fertilised egg, simple and almost homogeneous in appearance, is rich with the whole complexity of the future being. But there is nothing to show its extreme complexity and we no longer consider the egg as a miniature replica of the adult, containing all the organs in a preformed state.

The hypothesis of preformation has been replaced by the doctrine of epigenesis, which is not a theory but a series of facts. Systems, organs, progressively differentiate in time and space and we can observe in the embryo, at specific points, the formation of structures of which nothing had given us any reason to expect. Of course, all these differentiations have a cause and the links between them are slowly becoming known.

It is known that developing organs influence each other and, starting from the first rudiment the prospective fate of which is determined early, one can explain all the successive steps in development by means of successive actions and reactions. It is also known that these influences can be explained by the production of chemical substances, which can be extracted from certain determined rudiments known as organizers and made to react on other rudiments that remain to be determined. But how do these complex chemical substances fit into place when and where required? How are the first localisations chosen, positions responsible for the entire future organisation?

Behind each problem solved lies an unsolved one. And this one in particular is

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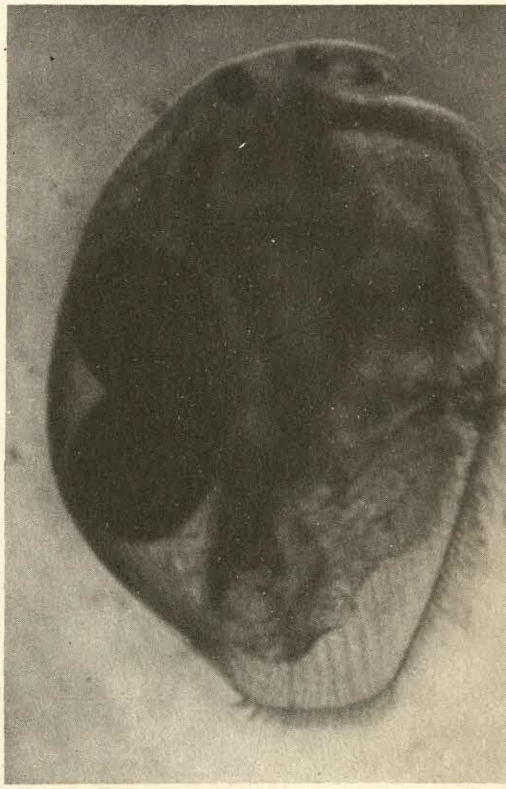
the eternal mystery: how can a homogeneous egg give us a heterogenous embryo? We know of course some of the reasons: Jenkinson, Ancel and Vintemberger have shown that specific factors enable the plane of bilateral symmetry and the two ventral and dorsal sides of a frog embryo to be determined at will. But we still remain surprised by the amazing complexity of an egg, a whole individual condensed within a single apparently simple unit.

Life sets many other general problems; it is governed by many other almost general laws, and yet it is a fact that some of its creatures happen to escape from even the most general of laws. Thus the almost universal rule for cell division is mitosis, or indirect division, with its stereotyped mechanism, but some cells, including the most differentiated cells of higher organisms, divide by simple amitosis. Sexuality is an almost universal method of reproduction, and its sequence of events absolutely constant, but some species reproduce by parthenogenesis, that is to say, they develop from eggs without the entrance of a sperm. True, this phenomenon is a form of sexuality in which nearly all the steps preceding and accompanying fertilisation can be found: we can describe it as an imitation of fertilisation. But other organisms reproduce indefinitely by simple division or by budding, without any sexual manifestations.

Regeneration is a very general property belonging to living beings, decreasing from inferior to superior organisms; within one group, a species can regenerate an entire organ from a minute part separated from the whole, whereas a very similar species may have only very limited possibilities of regeneration.

The reader of this introduction may be disappointed to find so many problems which remain unsolved, so much uncertainty on vital questions. But he must not despair. He will find many positive results in this book, many concrete answers to our questions. Biology has made great progress within the last century and problems are constantly being solved. For biology this is still the golden era of science, similar to that which confronted Ampère and Faraday in physics. New techniques are always opening up new paths, methods of attacking new problems, and research workers have a vast and fertile field before them. But the author of this article felt bound to show how the science of life is not a closed subject and that many problems, even the most general ones, remain unsolved and that the sum of our ignorance far exceeds that of our knowledge.



Daphnia *or water-flea: certain species reproduce themselves by parthenogenesis, that is, by the division of unfertilised eggs.*

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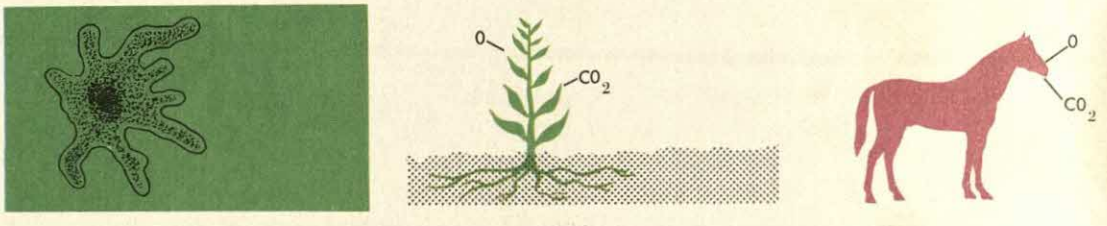
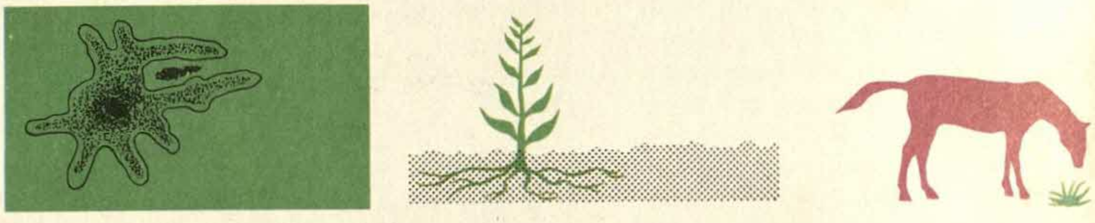
Characteristics of living beings

We are alive. What does this mean? The idea of life seems straightforward enough and yet the division between the inorganic and the living world is difficult to establish. Inert matter (a piece of furniture, a stone) is made up of chemical elements in the same way as a plant or an animal tissue. So what are the main characteristics which enable a biologist to define the subject of his research? The diagrams below give the answer. Whether it be a simple cell (an amoeba, for example) a plant or an animal, the presence of seven specific features makes it possible to qualify it as being alive: it breathes, it feeds, it excretes, it reproduces itself, it grows, it feels, and it moves.

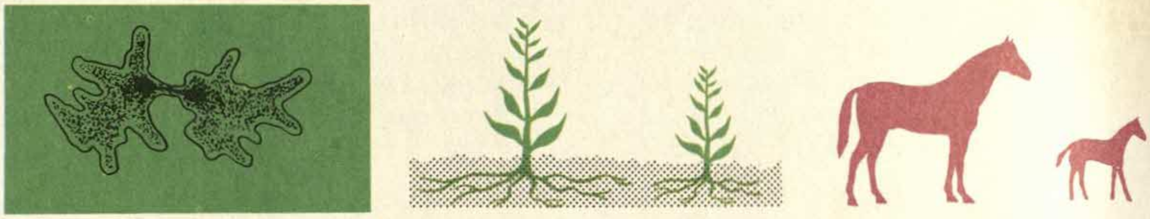
BREATHING: The amoeba, the plant, the animal, can only exist by absorbing oxygen and exhaling carbon dioxide.



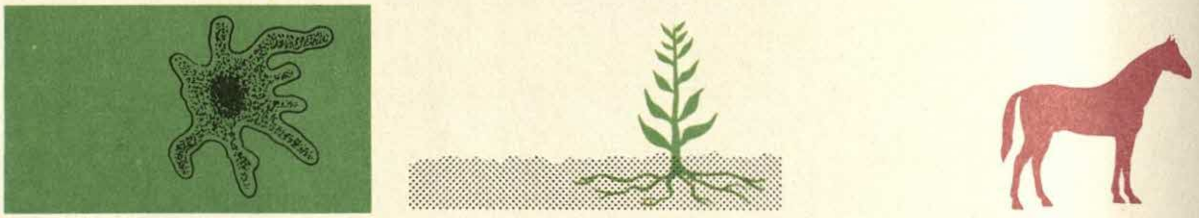
FEEDING AND EXCRETING: The amoeba by means of its pseudopodia, absorbs other organisms; the plant, through its roots, assimilates food, then exudes water (guttation) and gases; an animal eats and excretes.



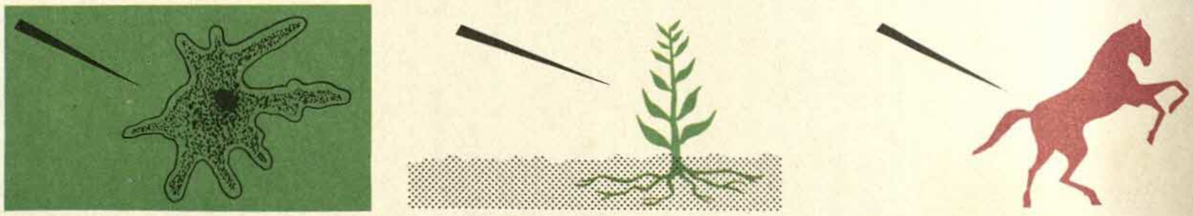
REPRODUCTION: The amoeba divides, the plant produces a seed which, when fertilised, creates another plant; the animal, after fecundation and a period of pregnancy, gives birth to another animal.



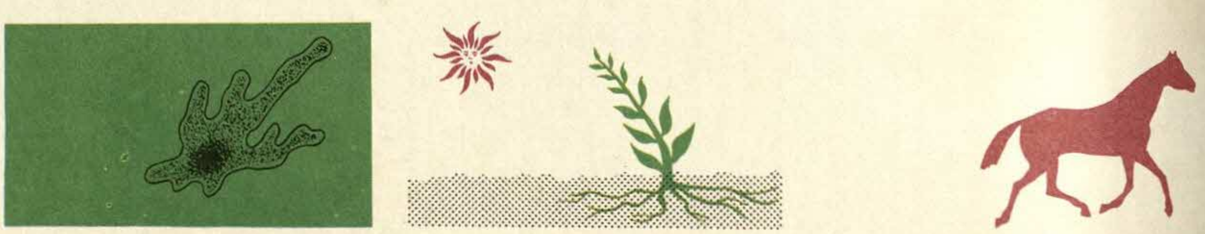
GROWTH: After division, the amoeba grows; so do plants and animals.



FEELING: An amoeba reacts to touch, some plants close if touched, an animal rears up when goaded.



MOVEMENT: The amoeba moves by means of its pseudopodia; the plant turns towards the sun (tropism); an animal can move freely.



The techniques of biological research

Philippe SENGEL

> *Only scientists can know the mind of a scientist; with its astonishing philosophical lack of curiosity, its specialisation, its almost childlike ignorance of problems not touching it directly, it nevertheless encompasses science, one of the rare adventures of humanity which has succeeded (even to the point of permitting a definition of the word success): whether we like it or not, it shapes part of the world of the future; it knows how to handle that most valuable of human tools, experiment, which makes the world tremble, controls life and, perhaps tomorrow, may even be able to imitate the mind.*
>
> RÉMY CHAUVIN

Man wants to understand the mechanism of the world. He questions the universe around him and, of all the mysteries he observes, life itself seems to him the most incomprehensible. He expects biology, the science of life, to give him the answers to such questions as: 'What is life?' 'From where does it come?' 'What laws govern the existence of all the living creatures which people the Earth?'

The explanations which the biologist is able to give today are still far from satisfying. And yet the sum total of our knowledge is already so vast that it has to be distributed over dozens of specialised disciplines each of which occupies numerous researchers all over the world. How has this knowledge been acquired? What means has biology at its disposal? What methods are used in biological research laboratories? Unlike physics and chemistry laboratories which require considerable and increasingly complex technical equipment, a biological laboratory needs only the simplest instruments, since its main complexity lies in the substance which it studies: living matter.

In fact, when studying living beings, man is confronted by a machine which 'works' without his intervention. First of all, then, he must *observe* and describe in as much details as possible all those characteristics which distinguish life from inorganic matter: this is the first step in any research.

Pure observation

Without magnifying instruments such as microscopes, the fine structure of living beings would have remained forever invisible and inaccessible to observation. At the minimum distance for clear vision (10 inches) the human eye can at best distinguish two objects which are separated by 0·003 inches. In other words, the human eye has a discriminating power of 0·003 inches. But cells in the tissues of mammals, for example, are much smaller since they measure only about 0·01 mm (0·00039 inches). They are completely invisible to the naked eye.

The microscope gives man an enlarged image of the objects he wishes to study and the degree of enlargement depends upon the kind of apparatus he uses. From the magnifying glass to the electron microscope there is a vast range of instruments with resolving powers which give precise observations at all levels of the living machine. In some favourable cases, the highest magnifications of the electron microscope can already reveal the molecular structure of a living being.[1]

There are basically two kinds of microscopes currently used in biological laboratories: the ordinary microscope (we shall see that this can be called the photon microscope) and the electron microscope. The former is incapable of enlargements beyond 2500, whereas the latter can give magnifications of anything from 5000 to 200,000 times.

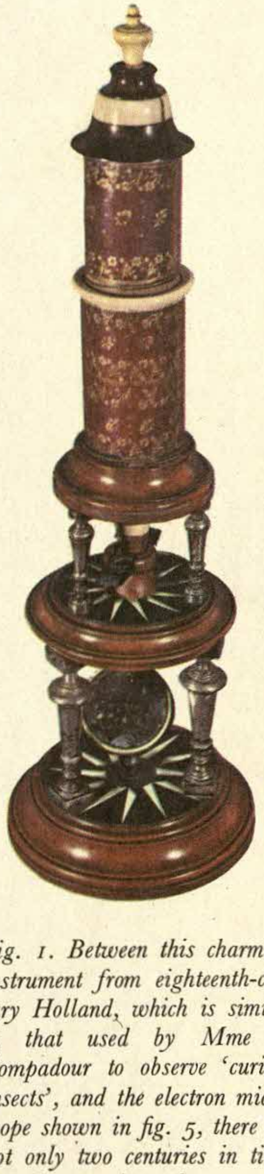


Fig. 1. Between this charming instrument from eighteenth-century Holland, which is similar to that used by Mme de Pompadour to observe 'curious insects', and the electron microscope shown in fig. 5, there are not only two centuries in time, but the creation of a research system which has opened a new world to biologists (photo Lod).

The photon microscope

We shall see that the basic principles of microscopy are the same for both kinds of microscope. But to simplify matters, let us first consider the elements of an ordinary microscope and what occurs when an object is examined under it. Figure 2 represents diagrammatically the main parts of a microscope. A source S throws luminous rays on to a first lens C, called the condenser, which concentrates the beam of light onto the object O. A second lens L, called the objective, gives an enlarged image, I, of the object. A third lens L′, called the eyepiece, focuses part of this image and enlarges it a second time into a second image I′. It is this image that the observer can examine when he looks into the eyepiece.

Let us study more closely the source of light of our microscope; for it is upon this that the maximum resolving power that can be attained ultimately depends. Like all rays, light can be considered as having both a corpuscular and undulatory nature. The source sends out corpuscles of light known as photons (hence the name given to the photon microscope) with which are associated waves whose wave-lengths vary from 0·0004 mm (violet) to 0·0008 mm (red). Thus if we wish to study with a photon microscope, a microbe of about the same order of size as the wavelength used, that is about 0·001 mm, it can only be illuminated by and reflect one or two waves at the most, so we shall have little more than a vague outline of the object and no details of its structure.

To illustrate this phenomena more vividly, let us give an example. Put your hand, with fingers wide apart, flat on the table (fig. 3) and then scatter some fine sand over both. Soon the whole table will be uniformly covered with sand except for the part hidden by your hand and when it is removed it will be seen that the grains of sand have clearly outlined its shape. Now try the same experiment with beads of 1 centimetre in diameter and the area covered by your hand will appear as a vaguely oval shape which shows neither the contour nor the number of fingers. Beads are too large to give a faithful reproduction of a hand.

In the same way, ordinary visible light is too coarse to illuminate objects as small as a microbe. So by its very nature light imposes an impassable limit of the order of 0·0004 mm on a photon microscope.

How can we obtain better resolving power and therefore greater enlargement? The answer is obvious. We must use fine sand. In other words, the source of our microscope must emit rays of a much smaller wavelength.

The use of ultraviolet rays, with wavelengths which are approximately twice as small as those of light (0·00027 mm) doubles the power of resolution and thrusts back the limits of the visible[2] to within 0·0002 mm.

But such enlargements are still inadequate to detect the finer details of cellular protoplasm. So we must have recourse to electrons, where the wavelengths associated with these particles may be as much as 100,000 times smaller than those of light.[3]

The electron microscope

In 1926, a German physicist, Hans Busch, conceived of a device which could deviate and focus a beam of electrons just as a glass lens deviates a ray of light. An electron is a particle charged with negative electricity and, to bend its trajectory, it need only be submitted to an electric or magnetic field.

Max Knoll, E. Ruska and B. von Borries, the first to make an electronic microscope, used this property to create electrostatic or electromagnetic 'lenses' which, when placed in the path of electrons, play the same part as the glass lenses of a photon microscope.

We shall now see the profound resemblance between the two types of microscope (fig. 4). The source of an electron microscope is a cathode K emitting a flow of electrons[4] and these are accelerated by the difference in potential applied between the cathode and the anode A (the cathode-anode unit is the counterpart of the light sources of

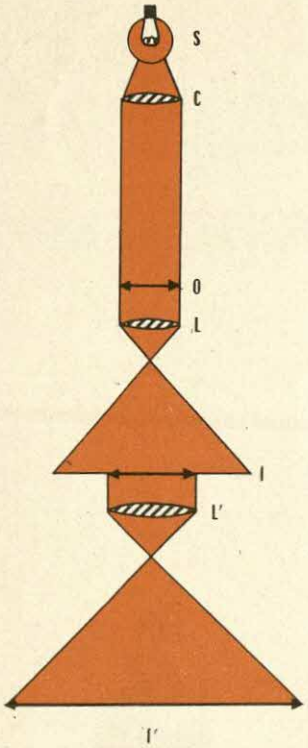


Fig. 2. Schematic diagram of the photon microscope. S: *source;* C: *condenser;* O: *object;* L: *objective;* L′: *eyepiece;* I: *primary image;* I′: *final image.*

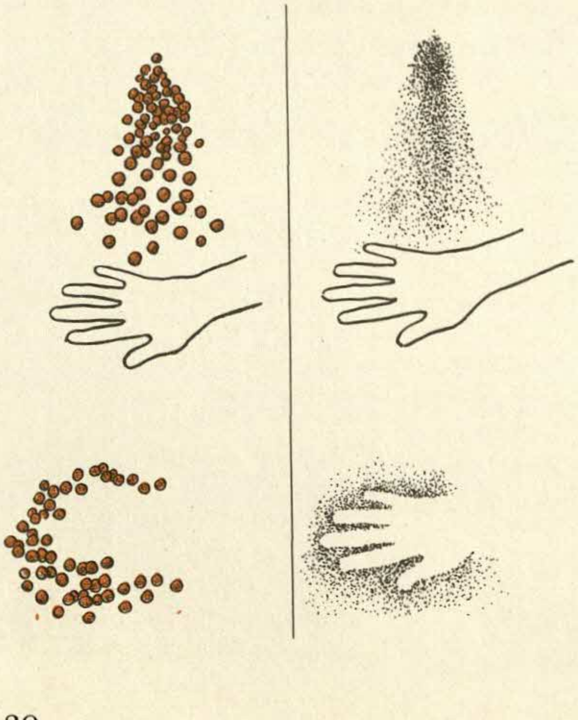


Fig. 3. Just as a sprinkling of fine sand shows up the shape of the hand better than one of large beads, so a flow of electrons (with associated wave-lengths 100,000 times shorter than the wave-lengths of light) gives greater resolving power than a beam of light.

(1) *The passage of ferritin molecules through cellular membranes has been studied by the electron microscope which reveals the stages of their metabolism and the part they play in the haemoglobin cycle of iron.*

(2) *Ultraviolet rays are not visible to the human eye, but are capable of making an impression on a photographic emulsion which replaces the eye in the microscope eyepiece.*

the photon microscope). If this difference in potential is 100,000 volts (a normal current for modern apparatus), the electrons will reach the anode at a speed of 164,000 kms/sec (more than half the speed of light).

The formula given above[3] enables us to calculate the wave-length λ associated with the electron travelling at a given speed, if we know that the mass of the electron, at this speed, is approximately $1 \cdot 09 \times 10^{-27}$) gm (or 0,000 000 000 000 000 000 000 000 001 09 gm). We find that $\lambda = 0 \cdot 000\ 000\ 0037$ mm, a wave-length one hundred thousand times shorter than that of light. The anode has a tiny central hole cut in it through which the electrons rush and are subsequently concentrated on the object by a first magnetic condenser lens C. The flow of electrons passes through the object O, being so to speak 'modelled' by the structure of the object. A second magnetic lens L acts as the objective and gives an enlarged image of it, I. The magnetic lens L′ then focusses on a small portion of this image and enlarges it yet again until the final image I′ is projected on a screen rendered fluorescent by the electronic bombardment. The observer can then study directly this fluorescent image or replace the screen by a photographic emulsion sensitive to electrons which will give a lasting document of the object examined.

But if the electron microscope can give these immensely greater enlargements, it does not enable living cells to be seen as can be done with a photon microscope. As we have seen,[4] electrons can only move *in vacuo*, so the object must necessarily be placed in a vacuum before it can be studied (fig. 5). It must also be extremely thin, less than 0·0002 mm (much less than the average thickness of a living cell) otherwise the electrons would be unable to penetrate it and it would remain opaque. In order to obtain suitably contrasting images, the tissues are impregnated with a heavy metal (generally osmium) which has the property of stopping electrons. Osmium is accumulated selectively by some protoplasmic structures and not by others and therefore the parts which are strongly charged with osmium and described as osmiophilic will oppose a more or less impassable barrier to the electrons being observed on the screen as black or grey. The parts of the protoplasm free from osmium, the osmiophobic parts, show up on the screen as luminous surfaces. So, in fact, the electron microscope enables us to examine a metal skeleton with differences in density which reflect the greater or lesser affinity of the cellular organelles for the metal (see photos, pages 38, 43, 48).

The electron microscope enables physicists to fulfil a long felt wish: for a long time they had dreamt of lenses with variable focal distance, that is to say, which are able to vary the enlargement in continuous fashion. With an ordinary microscope, in order to alter the enlargement, either the objective or the eyepiece must be changed, so that enlargements go by steps and not continuously. With the electron microscope, it is sufficient to vary the intensity of the current controlling the electromagnetic lenses for these to deflect an electronic beam more or less strongly, and therefore, within certain limits, the focal distance of magnetic lenses can assume all the desired values. It is also possible for the magnification to be increased or diminished progressively.

The power of resolution of the electron microscope depends on the difference in potential applied between cathode and anode. If the voltage is increased, the speed of the electrons increases. Therefore, according always to the fundamental formula of wave mechanics,[3] the associated wave-length decreases and the power of discrimination increases. But voltage acceleration cannot be increased indefinitely without meeting technical difficulties which soon become overwhelming.

There is another method of decreasing the wave-length: we can make use of corpuscles which are much heavier than electrons. At present a proton microscope is being developed in which the source gives out, as the name indicates, a beam of protons. The proton, one of the constituent parts of the atomic nucleus, is charged with positive electricity and, like the electron, can be focussed by electromagnetic lenses. It is 1837 times heavier than an electron and, at equal speed, the associated wave-length is 1837 times shorter than the wave which accompanies the electron. The proton microscope is still at the experimental stage and its employment in biology raises many difficulties, the most serious one being the extremely destructive effect of its rays on fragile protoplasmic substances. There can be little doubt, however, that soon the proton microscope will increase the range of observation instruments used by biologists.

Experimentation

The desire and the need to understand the mechanisms of vital phenomena arises from observation. But for this one must work on the living being and thereby modify some

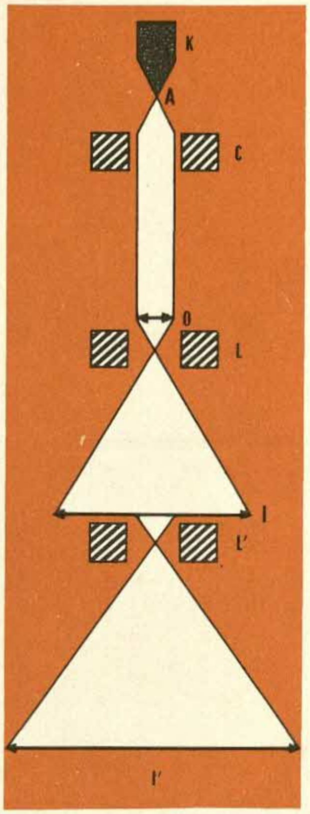


Fig. 4. Schematic diagram of an electron microscope. K: *cathode;* A: *anode;* C: *magnetic condenser;* O: *object;* L: *magnetic lens;* I: *enlarged image;* L′: *second lens;* Y: *final image.*

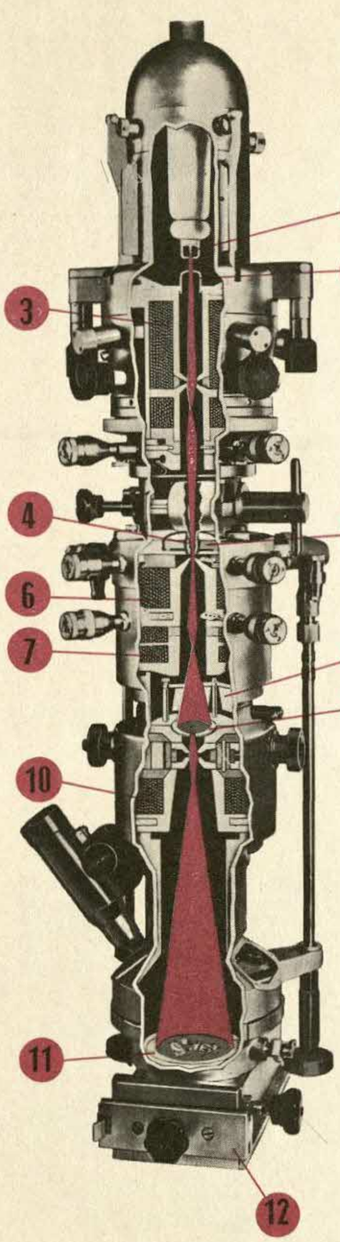
(3) *The fundamental formula of undulatory mechanics:*

$$\lambda = \frac{h}{mv}$$

expresses the relation between the mass m, the speed v of a particle and the associated wave-length λ; h is Planck's constant equal to $6 \cdot 6254 \times 10^{-27}$ erg-sec.

(4) *Electrons can only cover large distances when they encounter no obstacles in their path. That is why electrons are made to travel* in vacuo *in an electron microscope.*

(5) *Surgical instruments or injurious rays such as X-rays and ultraviolet rays or exposure to ultrasonic waves can be used.*



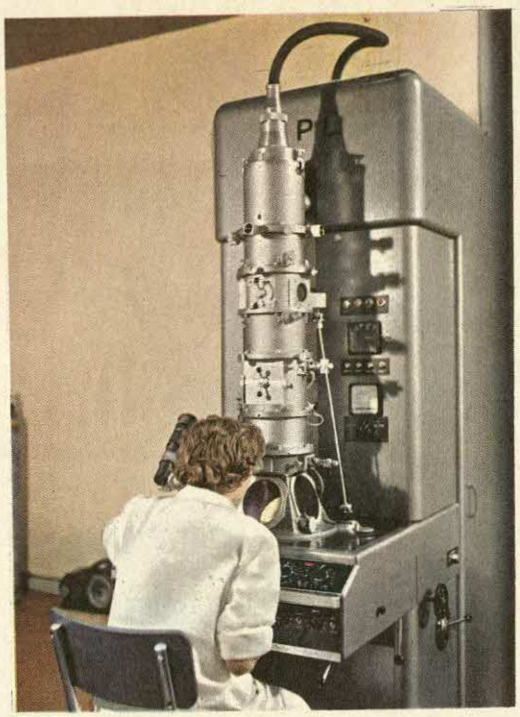


Fig. 5. External view of (right) an electron microscope and (left) model seen in section. 1: filament; 2: anode; 3: condenser; 4: beam of electrons; 5: microscopic slide; 6: objective winder; 7: first projective winding; 8: observation mirrors; 9: primary screen; 10: second projective winding; 11: fluorescent screen and image; 12: photographic chamber. (Left-hand photo: Siemens; right-hand photo: Atlas Photo).

of its living conditions. It is important, however, only to alter the variable under study and in particular the integrity of life must be respected. The biological experimenter remains above all an observer watching the reactions of living organisms to disturbances he has voluntarily caused.

There are many ways of acting on an animal or plant and amongst the most important and frequently used techniques are traumatic operations[5] (excisions, for example) and the injection of chemical substances (such as hormones[6]) in order to see their action on the organism.

Let us take as an example a cock and a hen whose external morphology differs in character: the male has a high and turgescent comb, whilst the female has a smaller and flabby one. We may then ask ourselves the question whether this distinguishing feature of the comb is really a sexual factor and, more specifically, whether it is associated with the presence of sexual glands. In order to find out we remove the male sex glands of the cock by surgical excision.

The comb immediately recedes. So it seems that the testes are essential for the cock to have a comb. Let us now make the test in reverse by grafting a fragment of the gland to the abdominal wall of a capon or castrated male. The comb recovers its original aspect.

It is known that the testes secrete a male hormone. Is it this hormone which is responsible for the comb's development? Testosterone injected into a capon gives us the answer; for the comb grows and gives a normal appearance to the castrate cock.

So three simple operations—excision, graft and injection—enable the experimenter to establish accurately the part played by testicular hormone in the development and appearance of the cock.[9]

The chorio-allantoic graft

Let us now consider the technical details of two of the commonest methods used in biological laboratories: the chorio-allantoic graft and culture *in vitro*. Both methods aim at isolating an organ or embryonic organ so as to remove it from the complex influences of the organism to which it belongs. These methods are most used in embryology, the study of the formation and development of living beings.

After three days' incubation, the embryo of a chick begins to show on either side of the body two small growths called limb buds (fig. 6a) and during its subsequent development, the front bud will become a wing, the rear one a foot. What will such a bud become if isolated from the organism? What are its potentialities of differentiation? We want to discover whether this bud after three days of incubation is already determined in such a way as to give rise to a complete limb with skeleton, muscles, and skin, or whether, for its subsequent normal development, it is necessary to maintain its normal relationship with the side wall of the embryo.

The chorio-allantois[10] of the nine-day old chick embryo, which is rich in blood-vessels, offers ideal conditions for the implantation and survival of an isolated embryonic organ such as the limb bud.

Some eggs are put into an incubator nine days before the experiment and these will act as hosts or grafting media. Other eggs, put into incubators three days before the operation, will serve as scions. The experiment is carried out in three stages:[11]

(*a*) *preparing the grafting medium*

Using scissors and pincers, a hole is made

(6) Hormone: *a substance secreted by an endocrine gland and circulated in the bloodstream which helps to maintain the equilibrium of organisms, regulate their growth, determine, at least partially, their form and to co-ordinate their functions.*

(7) Testosterone: *a synthetic crystallised hormone, chemical in nature, and with similar, if not identical, properties to those of normal testicular hormone.*

in the eggshell to expose the chorio-allantoic membrane (fig. 6b).

(b) preparing the donor

The three-day old embryo is taken out of its shell by means of a special spatula; it is placed into a small glass vessel containing warm physiological solution, then pinned on to the black paraffin lining the glass vessel. To separate the wing bud, the operator uses a pair of very fine scissors.

(c) transplantation and implantation

The bud is then taken in a transplantation spatula and placed on the host's chorio-allantoic membrane. It is inserted into a slit cut in the thickness of the membrane, where it remains, thanks to the elasticity of the chorionic tissue (fig. 6c).

The hole in the egg acting as a medium is then closed with a thin sheet of mica, sealed with paraffin, and the egg replaced in the incubator at 38°C.

Twenty-four hours later the graft is solidly implanted in the membrane and is soon supplied by a network of capillaries passing from one to the other. Nine or ten days after the operation, the host is sacrificed and, on its chorio-allantoic membrane, a perfectly well-developed wing is found (fig. 6d).

The answer to our question is therefore that the wing-bud of the three-day embryo chick is already set to supply a complete wing. At this stage it is already capable of self-differentiation when isolated from the parent organism. The results furnish an important element in the understanding of the development of an organ such as this.

These experiments were first carried out by Murray and Huxley (1925). The experiment gives the same result with the bud of any other organ.

Culture in vitro

Culture *in vitro* is another way of isolating part of a living being and studying it under simple experimental conditions which it is impossible to obtain in the whole organism and which are capable of being selected and varied at will by the biologist. This is the considerable advantage afforded by culture *in vitro* as compared with the graft, even if the survival of the tissue culture is sometimes more precarious than that of the scion.

If one wishes to study, for example, the multiplication of cells and to determine the factors which can stimulate or slow it up,

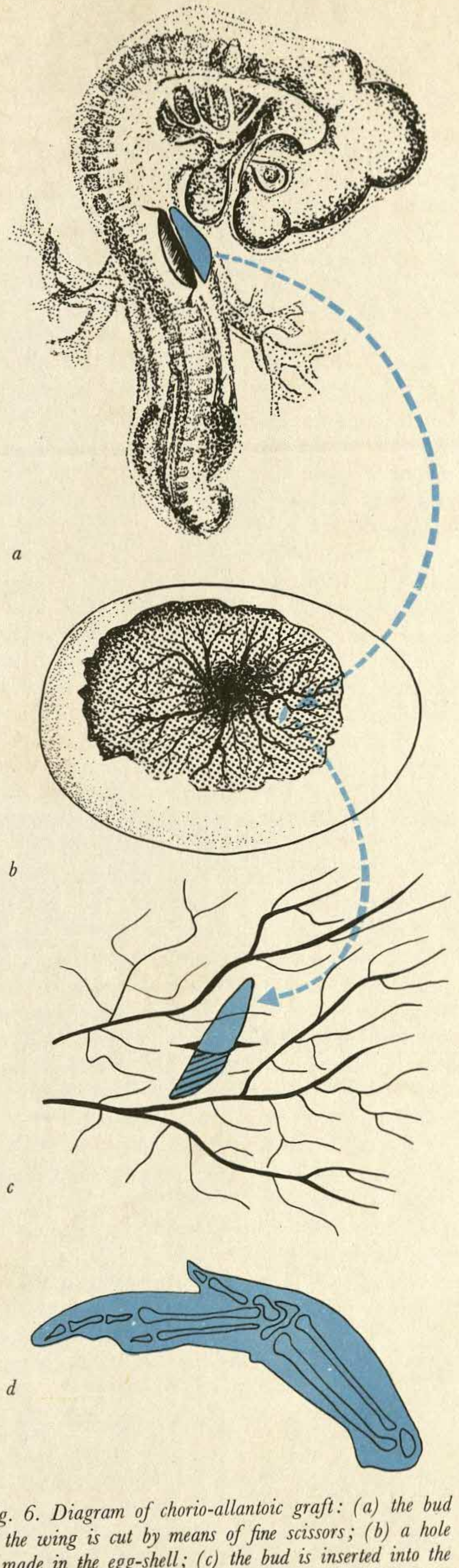


Fig. 6. Diagram of chorio-allantoic graft: (a) the bud of the wing is cut by means of fine scissors; (b) a hole is made in the egg-shell; (c) the bud is inserted into the chorio-allantoic membrane of the host; (d) it develops almost normally and forms a well shaped wing.

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(8) Castrate: *an animal deprived of its genital glands.*

(9) *These results are mainly from experiments carried out by A. Pézard from 1918 to 1922.*

(10) Chorio-allantois: *an organ of the chicken embryo which is basically respiratory in function and formed by the coming together of two embyronic organs: the allantois and the chorion.*

(11) *Generally speaking, sterilised micro-surgical instruments are used to avoid the introduction of any infectious germs into the egg.*

(12) *The culture medium is called 'natural' when the nutriment it contains, apart from sugared physiological solution, is composed of organic liquids (blood plasma, serum, extract of embryos) of indeterminate chemical composition. It is called 'synthetic' when all its components (amino acids, sugars, peptones, etc.) are chemically defined.*

(13) *All handling is done in sterile conditions. The atmosphere in the laboratory is freed from infectious germs by using ultraviolet rays, washing the benches in 90% alcohol, and sterilising all instruments and glass-ware in the autoclave or oven at 160° C for at least an hour.*

(14) *There are many variations in technique to the culture* in vitro *just described. Some are specially adapted to the culture of isolated cells or colonies of cells, others favour the cultivation of complete organs. The first are called 'histotypical', the others 'organotypical'.*

culture *in vitro* is the technique of choice, as it offers innumerable possibilities to the investigator.

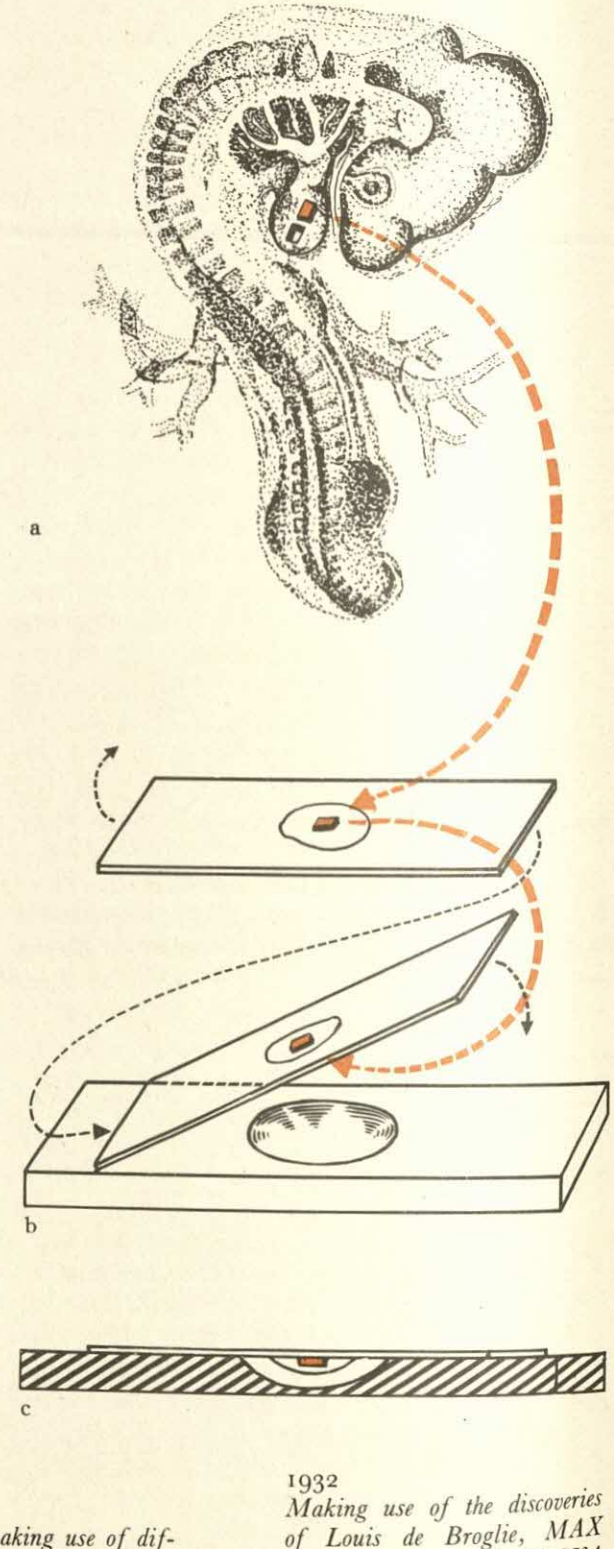
The heart cells of a chicken embryo are particularly favourable material for such studies. A fragment of the heart of a chicken's embryo is removed (fig. 7a) and placed on a glass slide in a drop of nutritive physiological solution[12] (fig. 2b). The slide is then turned upside down and placed on a glass sheet with sufficient concavity to hold the drop without squashing it (fig. 7c). Drying up of the culture and infections[13] are avoided by hermetically sealing the slide against the concave glass with paraffin. Slides on which the explants are placed are then put into an incubator at 38° C and, hour by hour, day by day, the investigator follows their development.

If the culture medium is favourable to the survival and proliferation of the cells, soon, around the original fragment of heart, there will appear a ring of migratory cells which, after detaching themselves from the explant, come to rest on the thin plate covering the glass slide. Under the microscope, one can see that these cells are actively dividing so that the surface-area of the culture increases considerably. The diameter of the ring thus gives us a means of measuring the capacity for cell proliferation in the medium tested.

It is easy to understand that by varying the chemical composition of the culture environment, the operator is able to study many problems concerning the nutrition and metabolism of cells, tissues and organs.[14] Here are a few of the questions to which culture *in vitro* can supply definite answers: What are the essential foods required for survival and growth? How do such important chemical substances as vitamins and hormones act? Are there factors which stimulate cellular division and others which produce specific differentiations (as, for example, the transformation, during development, of ordinary connective tissue into cartilaginous cells)?

Many problems have already been solved thanks to this valuable technique and there is little doubt that it will continue to give us knowledge of the nature of life in the future.

Fig. 7. Diagram showing the technique of tissue-culture. (a) A portion of the heart-rudiment is excised from a chick embryo and placed on a glass slide in a drop of nutritive physiological solution; (b) the slide is reversed and placed on another with a hollow-ground well which accommodates the piece of tissue in its culture medium; (c) sectional view of the preparation which can then be studied under the microscope.



HISTORICAL NOTES

1590
JANSEN built an optical apparatus made of two lenses: one which was biconvex, served as objective, the other, which was biconcave, as eye-piece. The 'microscope', thus named by the Greek Demiscianus in 1614, was invented.

LEEUWENHOEK (1632–1723) used magnifying glasses and microscopes made by himself. With the help of these devices, he discovered the blood corpuscles, the infusoria and many micro-organisms.

With ROBERT HOOKE (1635–1703) the microscope came into the field of observation and scientific research. Hooke illuminated the objects under observation by means of an extra condenser lens.

1668
DIVINI invented the convex-plane eyepiece which gave clearer images than the Jansens eyepiece.

1712
HERTEL perfected the microscope by adding a lighting mirror.

1784
JAN INGEN-HOUSS had the idea of covering the object he wished to observe with a fine slide of glass. He thus discovered the advantage, for microscopic study, of thin, transparent preparations.

From 1757 to 1812
DOLLOND and FRAUNHOFER made 'achromatic' lenses and thus did away with the coloured luminous fringes and distortions of optical systems used up till then.

1827
AMICI invented the 'immersion objective' which increased the resolving power of the microscope.

1857
VON GERLACH perfected a colouring technique for microscopic preparations. This was the beginning of all present-day histological staining techniques.

From 1866 to 1888 ABBÉ founded modern microscopic optics by discovering the laws and techniques on which the construction of present-day microscopes rests.

1903
SIEDENTOPF and ZSIGMONDY extended the limits of the visible by their conception and construction of the 'ultramicroscope', making use of diffraction images seen when a beam of light is perpendicular to the axis of vision.

1932
Making use of the discoveries of Louis de Broglie, MAX KNOLL, ERNST RUSKA and B. VON BORRIES introduced the electron microscope.

The first thing that strikes Man when he contemplates his own being is his body, a portion of matter that is entirely his own. But in order truly to understand the nature of this portion, he must compare himself with all that is above and all that is below him, and thus recognise his inevitable limitations.

PASCAL

PART ONE

Life on a molecular plane

We have just described some of the chief methods used in the biological laboratory, but there are others, more specialised and less commonly employed, which can be applied to the study of function and the chemical structure of living matter. This application of chemical methods to the study of living organisms is known as biochemistry.

In the first part of this volume the reader will be introduced to some of the fundamental knowledge that has already been acquired by the application of biochemical techniques. What is life, in terms of molecules and atoms? The basic substance of the living cell is protoplasm, composed of organic and inorganic chemicals, in which incessant molecular activity is taking place. Whilst some molecules are built up, others are broken down, and so there are continuous energy exchanges both within the cell and between the cell and its environment. This activity, which is characteristic of all living matter and distinguishes it from inert material, is initiated and controlled by extremely important substances—the enzymes and nucleic acids.

How is a living organism constructed out of these chemical elements? In the second part of this book we shall describe the cells, tissues and organs which go to make up the living animal or plant. The cell is the smallest part of an organism capable of independent existence. It is composed of a central nucleus, which contains the hereditary characters, and the surrounding cytoplasm in which the essential metabolic processes take place, the whole being surrounded by a membrane which separates the cell from its environment. There are many varieties of cells which differ in size, shape, chemical composition and function; but those of the same type are grouped together to form tissues. Epithelial tissues, for example, are made up of cells which are joined together in sheets, whilst in another type of tissue, known as mesenchyme, the cells are loosely arranged and separated by intercellular spaces, being connected to each other only by means of cytoplasmic bridges. Epithelium and mesenchyme together form more complex structures, the organs, the functioning of which is dependent on the co-ordinated activity of the constituent tissues. Each organ has a specific function, and the different organs are dependent on each other to such an extent that the life of an individual would be impossible without the controlling influence of extremely complex chemical and nervous mechanisms.

Ageing and eventually death are the inevitable fate of all living organisms, and in the third part of this volume it will be seen that, at the end of its period of growth, the organism progresses slowly and imperceptibly towards its end. Nevertheless, there are some cells which do not age as rapidly as others and retain their potentialities for growth and division. Yet other cells which are associated with reproduction can be said not to die at all, for they hand on from generation to generation the specific hereditary characters and thus ensure the continuity of the species.

The fertilized egg, formed by the union of a reproductive cell from each parent, is quite unique; it is the beginning of the growth and development of an entirely new organism. The fourth section of this book describes the remarkable stages of embryological development which bridge the gap between egg and adult. The reader will witness the establishment of bilateral symmetry in the future animal, and, during the process of early development, the formation of the three embryonic layers (ectoderm, mesoderm and endoderm) which characterise the embryos of all vertebrates and many invertebrates. He will also learn about the elaboration of tissues and organs, and the appearance in embryonic cells of chemical substances which are specific to the adult.

From living being to chemical formula

1. All living beings are made up of a number of organs, each of which has a specialised function. The liver can be taken as an illustration to show the composition of living matter.

2. The liver is made up of a large number of polygonal lobules which are permeated by blood vessels and bile ducts.

3. The cells in each lobule have many faces and are closely packed together.

4. In each cell one can distinguish the nucleus and the surrounding cytoplasm.

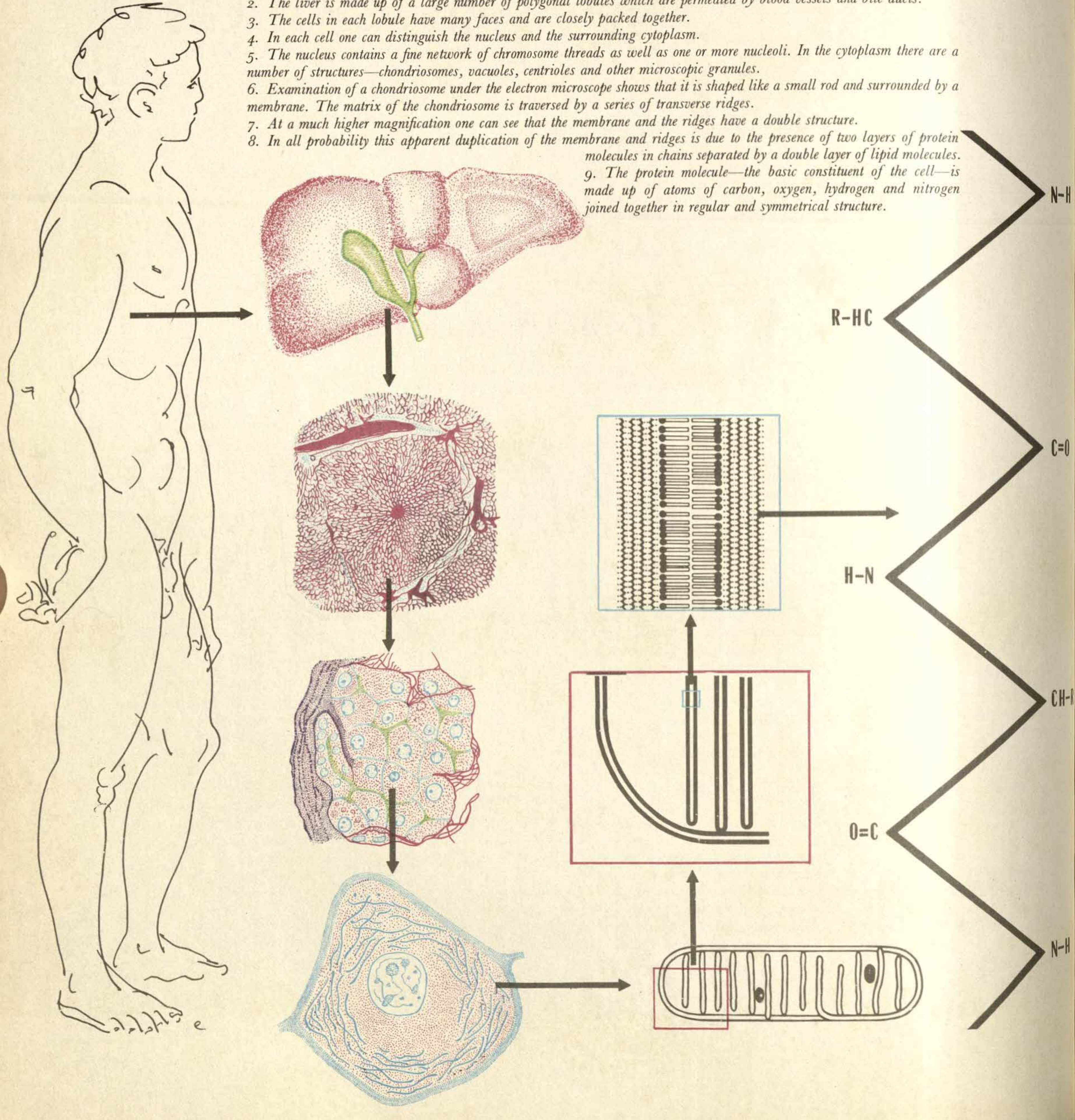
5. The nucleus contains a fine network of chromosome threads as well as one or more nucleoli. In the cytoplasm there are a number of structures—chondriosomes, vacuoles, centrioles and other microscopic granules.

6. Examination of a chondriosome under the electron microscope shows that it is shaped like a small rod and surrounded by a membrane. The matrix of the chondriosome is traversed by a series of transverse ridges.

7. At a much higher magnification one can see that the membrane and the ridges have a double structure.

8. In all probability this apparent duplication of the membrane and ridges is due to the presence of two layers of protein molecules in chains separated by a double layer of lipid molecules.

9. The protein molecule—the basic constituent of the cell—is made up of atoms of carbon, oxygen, hydrogen and nitrogen joined together in regular and symmetrical structure.



The constituents of living matter

Philippe SENGEL

Nobody knows how life originated upon Earth and, although many theories have been advanced, we are as yet reasonably certain of one thing only, that life had its origin in the water.

Many living species have now colonised the dry land whilst a large number of animals and plants continue to live an aquatic existence. Yet for all of them the processes of life are intimately dependent on the presence of water, the principal constituent of cellular protoplasm.[1]

Water, the basic substance of life

The cellular protoplasm is the site of all those chemical reactions which sustain life, but in addition to water it contains many organic and inorganic substances which contribute to the development and maintenance of life.

The proportion of water, minerals and organic substances in living animals and plants

	Animal %	Plant %
Water	60	75
Minerals	4·3	2·5
Organic substances	35·7	22·5
made up of:		
carbohydrates	6·2	18
fats	11·7	0·5
proteins	17·8	4·0

An analysis of animal and plant protoplasm shows that 95 per cent of living matter is composed of four simple elements—oxygen, carbon, hydrogen and nitrogen and, together with eight others, these account for 99·99 per cent of the whole. In man these twelve elements are, in decreasing order of importance, oxygen, carbon, hydrogen, nitrogen, calcium, sulphur, phosphorus, sodium, potassium, chlorine, magnesium, and iron. In addition, there are many other substances present only in very small amounts which nevertheless play an extremely important role in the oxidation-reduction mechanisms of the cell.

The table shows how the different chemical constituents are distributed in plants and animals. There are three main varieties of organic substance in protoplasm; carbohydrates, or sugars; fats, or lipids; and proteins. The water in the cytoplasm exists in three forms; bound water, which forms part of the protein molecules and represents about 4 per cent of the total water in the cell; water in the protoplasmic colloids;[2] and capillary (or free) water.[3]

Fig. 1. Life first started in water (photo Lod).

(1) *See p. 59, 'From cell to tissue'.*

(2) *Colloids. A substance is in a colloidal state if, when dissolved in a liquid such as water, its particles measure 1–100 mμ. These particles are sometimes large single molecules (macromolecules), but more often they are aggregates of molecules. In a true solution the substance is distributed as molecules or as isolated ions with a dimension of about 0·1 mμ (see fig. 3).*

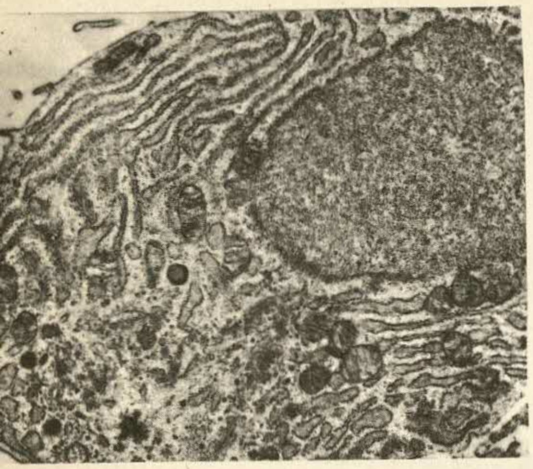


Fig. 2. The cell protoplasm is traversed by a system of filaments, membranes and canals dispersed throughout an aqueous medium (mag. × 9,300)(photo Dr Bessis).

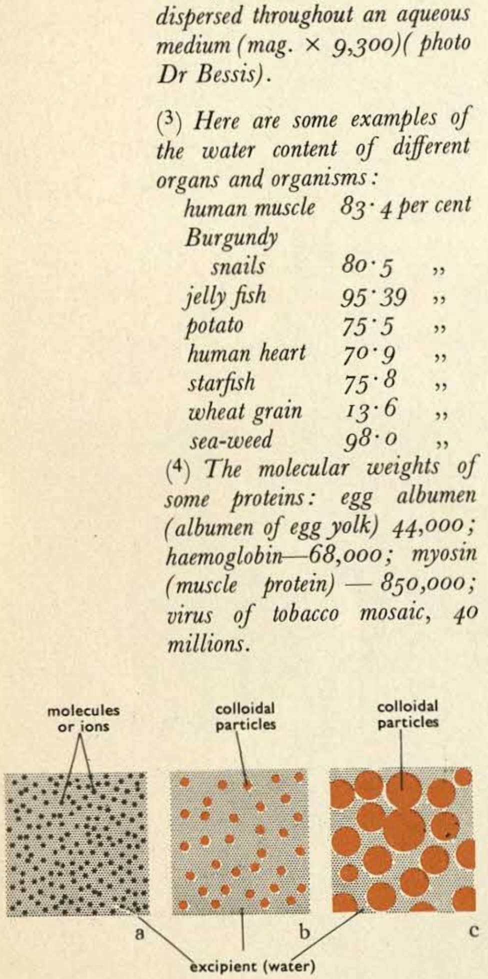


Fig. 3. Schematic representation of a true solution (a), a colloid in the fluid state or sol (b), and a colloid in the semi-solid state or gel (c). In (b) the colloid particles are of small size and contain only a little water; in (c) they are distended by the absorption of a large quantity of water.

Besides these constituents of the cell itself, there exist in the protoplasm minute quantities of compounds, derivatives of proteins, fats or carbohydrates, which exert a regulatory influence on cell metabolism and often act as catalysts. Some, like the hormones, are manufactured within the cell, whilst others, such as the vitamins, cannot be synthesised by the organism and have to be ingested in the food.

Proteins, fats, carbohydrates

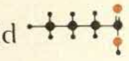
Later we shall see that living matter develops from a unique cell—the fertilised ovum—with its endowment of chemical substances derived from the parents. During embryonic life and after birth the materials necessary for growth and activity are brought to the organism in its food. Foodstuffs are ingested as complex substances—proteins, fats and carbohydrates—which are then digested in the alimentary tract and broken down into simpler chemicals which are able to pass through the cell membrane and into the cells. What is the composition of these substances?

Proteins are the most important constituents of the cells. They are known as quaternary compounds because they are made up principally of four elements, carbon, hydrogen, oxygen and nitrogen although they often contain phosphorus and sulphur as well. The protein molecules are large and complicated, containing many atoms, and thus tend to have a high molecular weight.[4] On hydrolysis, as for example by the digestive enzymes, they yield simpler substances, the amino-acids, which possess an organic acid group (—— COOH) and a basic amino group (—— NH_2).[5] Twenty-nine amino acids have so far been isolated and twenty of these are common to all living beings. The simplest amino-acid is glycine which has a radicle with only one atom of hydrogen. In the protein molecule, the amino-acids are joined together in a characteristic way known as the peptide linkage.[6]

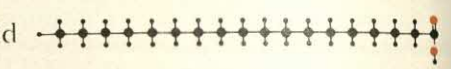
Apart from these simple proteins, which are composed solely of amino-acids, there are conjugated proteins which, on hydrolysis, liberate other substances in addition to amino-acids. Examples are milk casein and certain proteins of egg yolk which contain phosphoric acid (these are phosphoproteins); the ground substance of cartilage contains a glycoprotein (chondromucin) the molecule of which contains sugar; the greater part of cell nuclei is made up of nucleoproteins and these when hydrolysed, yield nucleic acids; finally, the haemoglobin of blood is a combination of a specific globin and a pigmented group which contains iron—and this is described as a chromoprotein.

The lipids are found in fats and are esters[7] of more or less complex alcohols with fatty acids of at least four carbon atoms. The two chief types of alcohol which enter into the composition of lipids are glycerol,[8] and the sterols, and the fats which contain them are tertiary lipids because they contain only carbon, oxygen and hydrogen. For example:

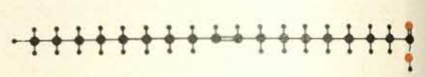
butyric acid



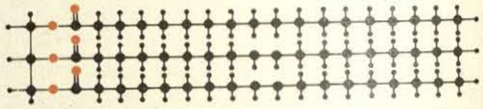
stearic acid



oleic acid



In the formation of the molecule of one of these lipids, a molecule of glycerol is esterified with three molecules of fatty acids and three molecules of water are liberated. This is the case, for example, with di-oleo-stearo-glyceride, which can be obtained from human fat:



Among the sterols, cholesterol is one of the most important in human metabolism, it is a tetracyclic alcohol and in human tissues one finds, in addition to free cholesterol, small quantities of cholesterol oleate (a steroid) which is produced by the esterification of one molecule of cholesterol with one of oleic acid. The sterols and steroids are widespread throughout nature and fulfil many important functions. Thus many hormones (the sex hormones and the hormones of the supra-renal cortex, for example) and some vitamins (e.g., vitamin D) are derived from sterols.

Apart from the tertiary, or simple, lipids there are compound lipids, of which the phospholipids are a good example; on hydrolysis they yield phosphoric acid as well as fatty acids and an alcohol. They are found principally in egg yolk and brain tissue.

Carbohydrates are also tertiary substances (composed of carbon, hydrogen and oxygen) but contain a reducing radicle (aldehydes) in addition to alcohol groups. It is important to distinguish between the simple sugars which cannot be hydrolysed, and therefore are not

(3) *Here are some examples of the water content of different organs and organisms:*

human muscle	*83·4*	*per cent*
Burgundy snails	*80·5*	,,
jelly fish	*95·39*	,,
potato	*75·5*	,,
human heart	*70·9*	,,
starfish	*75·8*	,,
wheat grain	*13·6*	,,
sea-weed	*98·0*	,,

(4) *The molecular weights of some proteins: egg albumen (albumen of egg yolk) 44,000; haemoglobin—68,000; myosin (muscle protein) — 850,000; virus of tobacco mosaic, 40 millions.*

broken down by digestion, and the compound sugars which can be hydrolysed to produce one or more simple sugars and perhaps eventually a non-carbohydrate fraction. Glucose[9] and ribose[10] are the most important simple sugars. Cane sugar, or sucrose, is a compound sugar and contains one molecule of fructose and one of glucose; and cellulose is a complex carbohydrate formed by an indeterminate number of glucose molecules arranged in a chain. Certain other complex carbohydrates yield substances which are not sugars; the nucleosides, for example, contain a non-sugar fraction which, in this case, is a nucleic acid.

All the substances in protoplasm are in contact with water. Those of small molecular weight are in true solution, but those with large molecules—the proteins, for instance—are in a colloidal state.[2] The colloids are responsible for the most obvious physical characteristic of protoplasm, its jelly-like appearance—more precisely it is a gel possessing special properties. At one moment it is firm like a paste, and at another it becomes relatively fluid and almost liquid; the change from one state to the other is reversible and can occur very rapidly indeed. Moreover, protoplasm is not a homogeneous gel; for throughout the aqueous medium it is permeated by filaments, membranes and small canals (fig. 2). It can absorb and eliminate water, thus explaining the continuous changes in viscosity and its contractility. This phenomenon is of considerable importance in all vital processes: muscular contraction, for example, is essentially due to a change in the distribution of water within the muscle fibres (fig. 3).

Metabolism, anabolism and catabolism

Within the protoplasmic gel relatively simple substances of low molecular weight having traversed the cell membrane, take part in multiple chemical reactions which are rapid and reversible being catalysed by enzymes. This is what is known as intermediary metabolism. Reactions of synthesis which allow the cell to manufacture its own specific material are called anabolic processes. Catabolism, on the other hand, occurs in the reverse direction when the large molecules in the cells and tissues are broken down to form waste products which will be eliminated.

Some of these reactions are accompanied by the spontaneous liberation of energy, whether chemical, electrical or in the form of heat (exogenous reactions). Others (endogenous reactions), by contrast, use up energy but they have to be coupled with exogenous reactions and both types of reaction are often initiated and powerfully accelerated by enzymes. Although the majority are reversible, a few are cyclic (substance A produces B which converts to C, and this is transformed back to A).

The different metabolic pathways of proteins, fats and carbohydrates converge in the production of certain substances—the 'cross roads' of metabolism—which can be influenced to change in one direction or the other under the influence of hormones or of chemical and physical agents. We shall see later that pyruvic acid, acetyl co-enzyme A, keto-glutaric acid, and oxaloacetic acid are intimately involved in the essential processes of cellular metabolism.

How is life possible?

The laws of thermodynamics teach us that energy is continuously being dissipated and that all molecular and atomic systems tend, in a fortuitous way, to arrange themselves in the simplest fashion and with a minimum energy content. This is known as entropy whereby 'randomness' throughout the universe is constantly increasing by an irreversible process; in other words, it is running down.

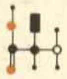
Now, life is governed by these laws and since the existence of living matter implies the creation and maintenance of cell structure, both building-up processes, one wonders how this is at all possible. Nevertheless, we have only to consider the vast number and variety of species that have colonised the surface of the earth over hundreds of millions of years to realise how remarkably well Nature has succeeded in her task.

It is the ability of living matter to utilise materials and energy in the environment in an orderly and constructive manner that has led to this success, and which distinguishes it from inert matter.

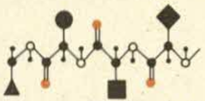
In the next chapter we shall see how living matter is able effectively to resist dissipation of energy by virtue of the action of enzymes or biological catalysts.

To summarise, we may say that the wonderful properties of the cell and the spectacular metamorphoses of living organisms shortly to be described are really no more than a chain of uninterrupted chemical reactions of very great complexity.

(5) *Amino-acids have the following general formula:*



(6) *Diagram of a peptide linkage:*



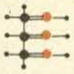
(7) Esters. *Produced by the combination of an alcohol radicle and an organic acid radicle with the elimination of one molecule of water, as follows:—*

$R-CH_2\ OH + H\ OOC-R'$

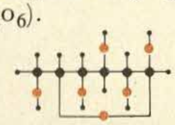
$\rightarrow R-CH_2-OOC-R' + H_2O$

This reaction is termed 'esterification'.

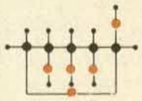
(8) Glycerol *is a tri-alcohol with three carbon atoms:*



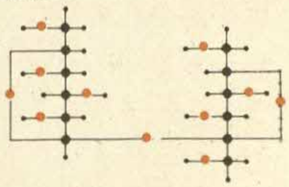
(9) *The formula of glucose:* ($C_6H_{12}O_6$).



(10) *The formula of ribose:* ($C_5H_{10}O_5$). *Ribose and one of its derivatives, deoxyribose, are sugars which enter into the composition of ribonucleic and deoxyribonucleic acids.*



(11) *The formula of sucrose:* ($C_{12}H_{22}O_{11}$). *This sugar is the result of the combination of two molecules of simple sugars with the elimination of one molecule of water.*



Explanation of the symbols:

● carbon

● oxygen

○ nitrogen

• hydrogen

■◆ ●▲▮ radicals of amino-acids

A chain of chemical reactions

Wiktor W. NOWINSKI

It is a platitude to say that the cell is a minute laboratory. If we look at it from the point of view of molecular dimensions, it is not as minute as it seems. Hofmeister once calculated that a liver cell consists of approximately 230,000,000,000 molecules including 200,000,000 molecules of proteins and fats, and if each molecule were the size of a brick, a structure could be built which would occupy an area of 2700 sq miles, with a height of 165 ft.

This world of molecules is in a state of permanent flux: various structures are broken down and re-synthesized, whilst other substances are built up. With the use of radioisotopes Schoenheimer was able to demonstrate that the chemical constituents of the body are in such a highly dynamic state. Half the total albumin in the blood of a dog is replaced in seven days, and all the globulins (among which is the one responsible for our resistance to infectious diseases) are replaced in only three days. What are the factors involved in these processes so essential for life? They are called enzymes or biological catalysts.

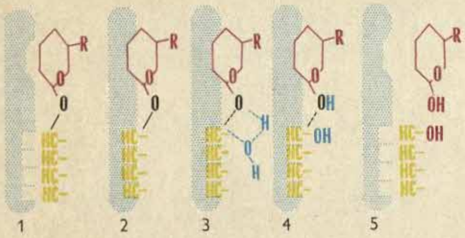


Fig. 1. Diagram of the mechanism involved in enzymatic activity: the splitting of a sugar-alcohol into sugar (red) and alcohol (yellow). The enzyme (blue) breaks the carbon-oxygen link (black) by attaching a hydrogen atom to the oxygen and an OH *(hydroxyl) group to the carbon, the source of hydrogen and the* OH *group being a molecule of water. (1) The substrate orientates itself in such a way that each of the two parts of its molecule faces one of the two active centres of the enzyme; (2) the molecule of substrate is attracted to and fixed by the enzyme; (3) a molecule of water becomes attached at the point of junction; (4) an* H *atom of this molecule attaches itself to the oxygen of the sugar, the* OH *group lying against the first carbon atom of the alcohol; (5) finally, a break occurs and the alcohol and sugar are released into the environment. The enzyme is then ready to repeat the reaction (hydrolysis) on another molecule of sugar.*

The function of enzymes

In catalysis, the active principles are the catalysts, substances which when present speed up the rate of chemical reactions and reappear unchanged at the end of these reactions, ready for further activity. An enzyme is a biological catalyst that influences the speed of biological reaction within the cell, or inside cavities of the organism. Enzymes do not perform miracles, but they speed up the reaction many times without the necessity of high pressures and high temperatures. As an example we may note that it takes an hour to build up the soft, tough, membrane of the hen's egg.

Without enzymes very many reactions (but not all) would have taken place anyway, but at such slow rates that they would not be of much use to the cell, where the processes run at speeds which can hardly be visualised. If we want to prepare acetaldehyde from ethyl alcohol in the laboratory, we have to oxidise the latter with powerful oxidizers; this slow procedure can be replaced by a quicker one, the passing of alcohol and air over red-hot copper or platinum, but this requires a very high temperature. Yeast contains an enzyme, alcohol dehydrogenase, which converts the acetaldehyde into ethyl alcohol. This activity is reversible and one molecule of alcohol dehydrogenase converts 43,000 molecules of alcohol into acetaldehyde in one minute. It is as though a man weighing 11 stone would consume in one minute an amount of food thirteen times greater than his own weight. With the improvement of methods of isolation and crystallisation, we have obtained more data on the speed of activity of enzymes, and there are others which work at even higher rates.

Our ancestors were well aware of the existence of enzymatic activity and used it in the form of fermentation. Primitive man was already brewing alcoholic drinks and the mythical nectar, drunk by the Greek gods, might have been prepared by fermentation of honey. However, the nature of this process was unknown until Pasteur in 1860 demonstrated that it was connected with living cells. The brothers Buchner obtained a cell-free extract of yeast which was still capable of producing alcohol by fermentation and thus opened a new way to the study of enzymes. The word 'enzyme' was introduced by Kuhne about 1880 to emphasise the *fermentative activity* of yeast and he generalised it as a concept by extending it to the already known activities of extracellular juices which, like pepsin, participate in the process of digestion.

The number of known enzymes today is about 700 and new ones are still being discovered. Of course, not all of them exist in man or in the vertebrates; many are found in unicellular organisms, particularly in bacteria. Just as there exists anatomically a common structural plan with specialised adaptations in certain species, there also exists biochemically a general metabolic plan or enzymatic pattern. Some of the most important discoveries in biochemical mechanisms of heredity were elucidated on moulds and bacteria.

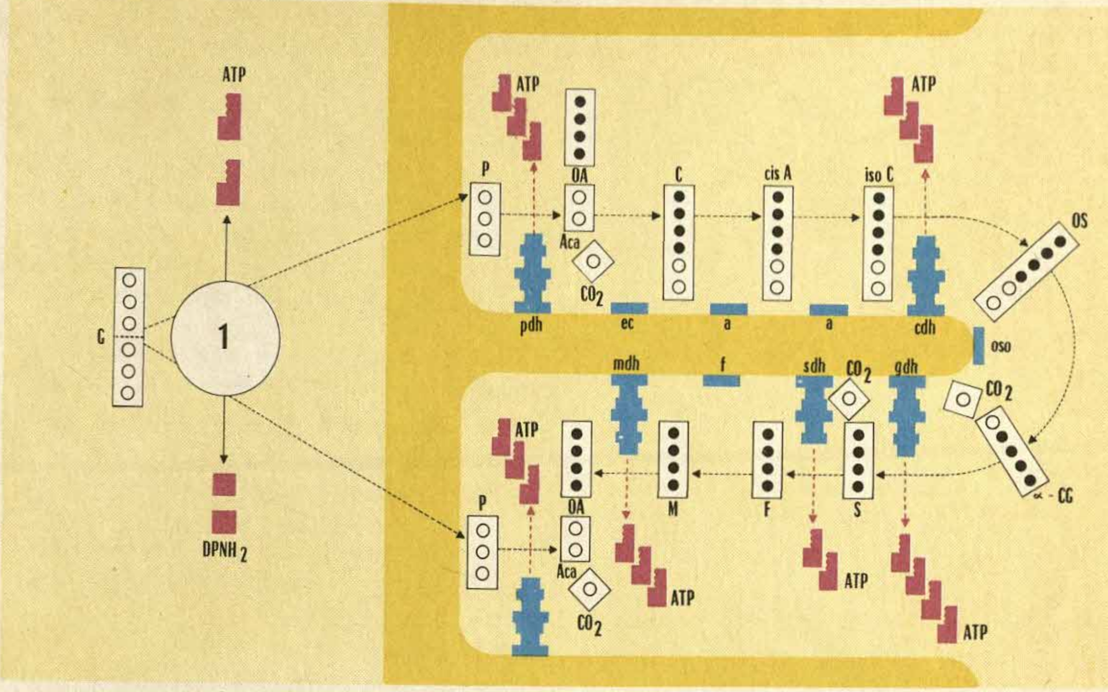


Fig. 2. The Krebs cycle taking place in the interior of a chondriosome (of which the membrane and three ridges are shown in yellow). A molecule of glucose (G) *is first broken down into two molecules of pyruvic acid by a chain of reactions catalysed by nine enzymes* (I)*; the energy released is stored in two molecules of* ATP *and two of* DPNH$_2$*; then the two molecules of pyruvic acid* (P)*, on entering the chondriosome, form with the aid of the enzyme pyruvate-dehydrogenase (pdh) a compound, acetylcoenzyme* A *(Aca), which combines with oxaloacetate* (OA) *originating in a preceding cycle (on the right) to produce a molecule of citrate* (C)*; then begins the real cycle, in which eight enzymes (blue) take part: a condensing enzyme (ec); aconitase (a); isocitrate-dehydrogenase (cdh); oxalosuccinate-carboxylase (osc); alpha-cetoglutarate-dehydrogenase (gdh); succinate-dehydrogenase (sdh); maleate-dehydrogenase (mdh); fumarase (f). The intermediate metabolites of the cycle are: citrate* (C)*; cis-aconite (cis-*A*); isocitrate (iso-*C*); oxalosuccinate* (OS)*; alphacetoglutarate* (CG)*; succinate* (S)*; fumarate* (F) *and maleate* (M)*; the production of energy (red) in the form of* ATP *takes place at different stages of the cycle; the breakdown of a molecule of glucose into carbon dioxide and water involves the synthesis of thirty-two molecules of* ATP *and two of* DPNH$_2$*; if oxygen is available, each molecule of* DPNH$_2$ *will produce three supplementary molecules of* ATP *by dehydrogenation (see p. 46); the enzymes participating in this production of energy are represented in the form of piles, the details of which are explained in fig. 3; at the end of the cycle, there is production of a molecule of oxaloacetate* (OA, *on the left), which begins a new cycle by combining with a new molecule of acetylcoenzyme* A*; the carbon atoms provided by the preceding cycle are represented by black circles, those provided by the molecule of glucose by white circles; they are eliminated one by one throughout the cycle in the form of carbon dioxide* (CO$_2$).

Enzymes are named according to the substance upon which they act (and which from now on we shall call *substrate*) by adding the suffix ‘ase’[1] to the name of the substrate. Thus, when we spoke of alcohol dehydrogenase, we meant an enzyme which acts upon ethyl alcohol by detaching two atoms of hydrogen and converting it into acetaldehyde. Enzymes are also classified into larger groups under the generic name of the activities which they perform, such as proteolytic enzymes (which split protein molecules), nucleases (which split nucleic acids), transferases (which transfer a certain chemical group from one substance to another), etc.

The structure of enzymes

Structurally, all enzymes are similar: they consist of two moieties, a protein and an active group attached to this protein. We have evidence that this group (if firmly attached to the protein it is called a ‘prosthetic group’; if loosely—a ‘coenzyme’) is connected with the activity of the enzyme, and that it is the place on the molecule where actual reactions take place. On the other hand, enzymes are proteins and possess their characteristics: they are denatured at a critical temperature (which is 56°C for thirty minutes[2]) and excessive acidity or alkalinity of the medium. Like very many proteins, they are antigenic, i.e., when injected into another species they induce formation of antibodies. On this principle immunity against micro-organisms is based. The fact that enzymes act as antigens opens certain new therapeutic possibilities. In many diseases there is an increased enzymatic activity which is detrimental to the normal functions of the cell: by injecting anti-sera, directed against specific enzymes, we may regulate their activities, either decreasing or inhibiting them totally. By using antibiotics, we kill certain strains, but also isolate the resistant strains, i.e., those upon which the antibiotics do not act. In this race between man's mind and the laws of nature, the latter will win, and we shall have no more active antibiotics. Specific anti-enzyme sera will then be of greatest importance.

The enzymatic activity depends upon a series of factors of which the most important are the proper degree of alkalinity (or acidity) of the medium, and the temperature. In both these cases there are optimal conditions at which an enzyme works at its highest rate but the optimal temperature may lie far beyond the normal temperature of the body from which the enzymes were extracted. The rate of enzymatic activity depends also upon the amount of the enzyme present, the amount of substrate, and last but not least, upon the accumulation of the products of reaction. In all these cases, the enzymatic activity follows the laws of thermodynamics. The majority of known enzymes work in both directions,[3] again according to the laws of thermodynamics, and thus the number of reactions which the 700 known enzymes can perform is almost doubled.

The bulk of knowledge on enzymatic activity is based on work done outside the organism, on enzymes extracted from the cell. Such isolated enzymes, in order to work at maximum activity, need optimal concentra-

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(1) *Nevertheless this rule does not apply to enzymes which were discovered a comparatively long time ago, such as pepsin, trypsin, etc. The word ‘pepsin’ was devised in 1835 by Théodore Schwann, to refer to a substance which brought about the activity of the gastric juice (from the Greek ‘pepsis’ meaning digestion).*

(2) *Certain enzymes, however, can withstand temperatures as high as 100°*C.

tions of alkalinity, substrate, co-factors, etc. It is not yet established whether these artificially created conditions are those which actually exist inside the living cell; we have good evidence that this is not so and that under normal conditions, enzymes work inside the cell at quite different rates. However, we do not know very much about these rates and their regulatory mechanisms in the cell, and it is up to the biochemistry of the future to elucidate them.

The role of enzymes

The enzymes in the cell are responsible for the living phenomena and their role is threefold: (1) to break down large molecules into small components in order to enable them to pass into or leave the cell;[4] (2) to synthesize new molecules, such as the proteins; and (3) to supply energy for the living processes of the cell.

The enzymes are also involved in various diseases. In cancers of the prostate, there is an increase of the enzyme, acid phosphatase, in the serum, and where there is suspicion of malignancy, the measurement of enzymatic activity helps to make the diagnosis. In coronary thrombosis, there is an increase in the oxalacetic-glutamic transaminase in the serum: this test is even more sensitive than the electrocardiogram. In inflammation of the pancreas or obstruction of the pancreatic duct, the serum amylase shows a considerably increased activity. These examples show how important enzymatic activity is in clinical diagnosis and this is one of the directions in which clinical enzymology is going

We know that there are enzymes that act directly upon the substrate, such as proteolytic enzymes, which split the bonds linking the amino acids in a protein molecule. But many enzymes need co-factors for their activity. Co-factors are substances of low molecular weight, often inorganic ions, and coenzymes; the latter, in the majority of cases, are built from vitamins, such as pyridoxal phosphate, which belongs to the group of B vitamins, diphosphopyridine nucleotide and triphosphopyridine nucleotide (DPN and TPN) which contain nicotinamide in their molecules.

We know that the molecule of an enzyme has a rough surface, indentations and grooves. Some of these are the active centres into which the molecule of substrate fits, as the hand fits a glove. By means of various chemical bonds and chemical forces the substrate attaches itself in several places and is thus held in a definite position. Then it is broken down or rearranged according to the type of activity of the enzyme. The fact that the chemical configuration of the substrate fits the active centre so closely explains also why the activity of some enzymes is very specific and that of others less so: in the former case, it is the *whole molecule* which has to have the necessary configuration; in the latter it is *only one group* on the molecule which has to attach itself. Fig. 1 shows an example of this concept.

In order to separate the two parts of a sugar, a breakage of the carbon-oxygen link has to be accomplished by attaching a hydrogen atom to the oxygen and a hydroxyl group to the carbon. The source of hydrogen and of the hydroxyl group is one molecule of water (see fig. 1).

Our example well illustrates the specificity of enzymatic activity: if, for instance, the alcohol moiety is composed of five carbon atoms they will not fit the active site. However, there is the possibility that the reaction can take place with three carbon atoms.

The energy requirements of the cell

The cell permanently requires energy: not only for enzymatic reactions, but also for muscle contraction, to supply electricity (for the electric organs of fishes), for the work of glands (to produce and expel the secretions) and for many other processes. However, the cell is a chemical machine and as such can use only chemical energy, just as an electric motor uses electric energy, or the steam engine energy from combustion.

In order to supply the cell with energy, there have evolved over millions of years certain most ingenious, but relatively simple mechanisms. This energy is taken from a relatively unlimited source—the sun: the sugar, glucose, is formed from carbon dioxide and water during the process of photosynthesis in the plant. The plant synthesizes glucose in such a way that in each molecule energy equivalent to about 680,000 calories is stored.[5] By consuming plants, the animal organism breaks down the glucose, releasing the energy.

This release of energy, however, does not take place spontaneously. Such a mechanism would be uneconomical, because most of it would be lost in the form of heat and unavailable for metabolic processes. Physicists are trying to devise machines which would supply us with atomic energy for our daily uses by releasing only small amounts. Nature, however, has already elaborated such a

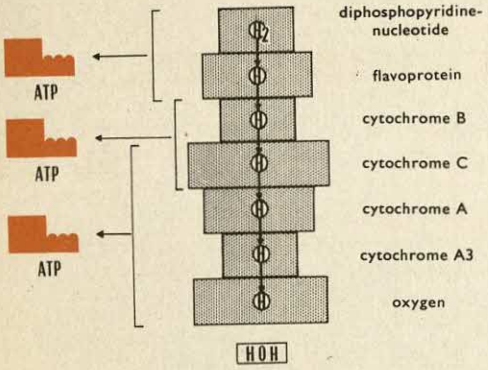

Fig. 3. Diagram representing the transfer of hydrogen which provides the various dehydrogenations in the Krebs cycle (detail of one of the 'stockpiles' of fig. 2). The coenzyme diphosphopyridine-nucleotide (DPN) *removes a hydrogen atom from the substrate; another coenzyme, flavo protein, picks up the hydrogen and transmits it to a chain of four cytochromes, the last of which gives it up to the oxygen thus forming water* (HOH).

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(3) *An enzyme capable of catalysing the transformation of substance* A *into substance* B *is in general equally capable of catalysing the reverse transformation of* B *into* A.

(4) *Immediately after the Second World War, a great number of under-nourished individuals lost partially or totally the ability to synthesize enzymes necessary to split proteins into their simple components, the amino acids and were unable to digest proteins. It therefore became evident that the body had to be supplied with these building-blocks from which the proteins could be synthesized.*

(5) *In the green parts of plants molecules of glucose are stored in the form of starch.*

(6) *The carbon dioxide of the acidic group* (COOH) *of organic acids is detached by enzymes; the hydrogen is transferred to combine with water but the* CO_2 *is eliminated through the lungs.*

system: the glucose molecule is broken down gradually to carbon dioxide and water, and in certain reactions of this process, chemical energy is released step by step, in small batches. The discovery by Krebs and Lipmann of this mechanism of energy release, was one of the most fascinating chapters of biochemistry.

Cellular respiration

Glucose is broken down in the cell and through a series of reactions forms two molecules of pyruvic acid, which are half as large as a glucose molecule. This pyruvic acid occupies a very important position in the metabolism of the cell, because there exist a number of alternate pathways or possibilities for its enzymatic conversion to other substances. Thus, if an amino-group is attached to it, it forms the important amino acid, alanine. By decarboxylation (or taking off carbon dioxide from the acidic group), it becomes acetaldehyde, from which ethyl alcohol is formed; via the formation of acetoacetic acid it can produce such substances as butyl and isopropyl alcohols or acetone. In the absence of oxygen, as in the case of intensive use of muscles, it is converted into lactic acid, and some think that the Marathon runner who ran to Athens with the news of Darius's defeat, died of acidosis provoked by accumulation of lactic acid in the blood rather than heart failure.

In the presence of oxygen, pyruvic acid combines with a co-factor called coenzyme A and forms acetyl coenzyme A, which in turn combines with a substance known as oxaloacetic acid to give citric acid. This citric acid molecule is already within the Krebs cycle and it undergoes a series of transformations, finally emerging in the form of oxaloacetic acid, which is again ready to react with another molecule of acetyl-coenzyme A, formed from pyruvic acid (see fig. 2).

The remarkable fact is that during these transformations, large amounts of chemical energy are released, energy which was originally stored in the glucose molecule. This energy is conserved in a special type of chemical bond, the so-called 'high energy phosphate bond'. The most important of these energy-rich compounds is adenosine triphosphate (ATP), which consists of a substance related to nucleic acids, adenosine linked with three phosphate groups:

$$\text{Adenosine} - \mathrm{P} \simeq \mathrm{P} \simeq \mathrm{P}$$

The two terminal phosphate bonds are those in which the high energy is stored (indicated by $\simeq$ = 'high energy bond') and each contains some 8,000 calories, or more than twice that of the bond which attaches the first phosphorus to adenosine. Such high energy phosphate bonds are readily broken by specific enzymes and the energy liberated is used by the cell. The two-phosphate form of such compounds, adenosine diphosphate (ADP), still contains one high energy phosphate bond.

There are only eight enzymes involved in the operation of the Krebs cycle within which the largest number of high energy phosphate bonds originate. The hydrogen atoms removed during a turn of the cycle are transferred to another system, the cytochrome system, discovered by David Keilin. This is the system through which the hydrogen, which comes from our food (substrate), ultimately passes and combines with oxygen to form water,[6] and liberates energy which is then stored in the high energy phosphate bond of the ATP by a process involving inorganic phosphate from the cell medium and adenosine diphosphate (see fig. 3).

These processes are the most important mechanisms in what is described as cellular respiration, because it involves the combustion of glucose which, at the end of the reaction, is completely transformed into carbon dioxide and water. However, glucose is not the only metabolite utilised in the phenomenon of respiration: proteins and fats, liberate amino-acids, fatty acids, and glycerol. All these substances, after a series of transformations, are equally capable of taking part in the Krebs cycle and thus liberating the energy locked up in their molecules.

Cellular respiration, then, may be regarded as a series of dehydrogenations and decarboxylations with oxygen intervening to take up the liberated hydrogen with the formation of carbon dioxide and water. It leads to the liberation of large quantities of chemical energy, which on the one hand goes to the production of heat, and on the other is available for mechanical work and growth.

Indeed, the cell is the centre of other metabolic reactions which are also catalysed by specific enzymes,[7] resulting in the synthesis of the constituents of living matter, proteins, fats, and carbohydrates.

To conclude, enzymes play a fundamental role in all living phenomena. It is through them that all the metabolic reactions take place which are indispensable to the production of living matter, of energy, and the major functions of the living organism. Enzymes, in each cell of the body, control an uninterrupted chain of chemical reactions which constitute the very essence of life.

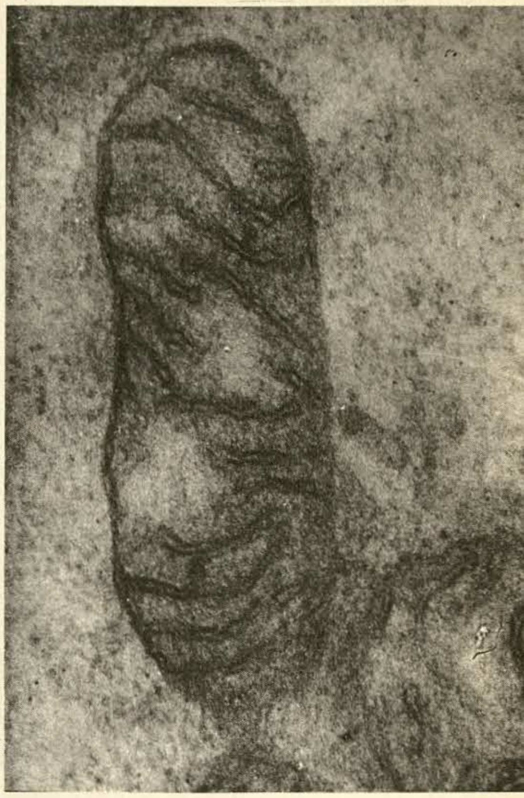
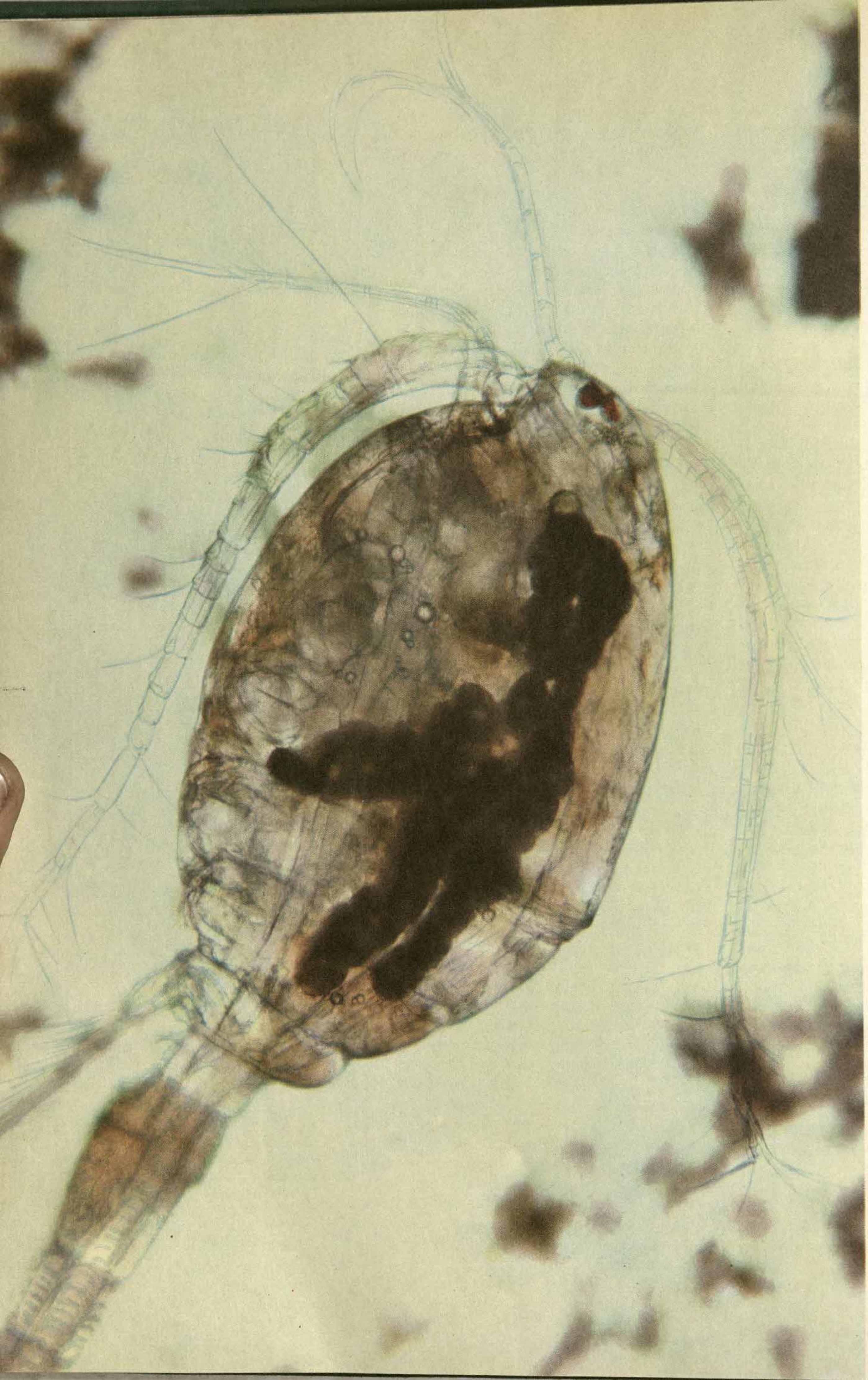


Fig. 4. It is within the chondriosomes or mitochondria of the cytoplasm that the Krebs cycle takes place. This shows a chondriosome under the electron microscope (photo Dr Bessis).

(7) *Where are the enzymes localised in the interior of the cell? We have seen that a large number are to be found in the mitochondria, but they also exist in the cytoplasm and microsomes. All the enzymes taking part in the Krebs cycle are localised in the mitochondria, but those which break up glucose into pyruvic acid are to be found in the cytoplasm. Thus the complete oxydation of glucose into carbon dioxide and water is carried out in two distinct parts of the cell: the first series of reactions occurring in the cytoplasm, the second in the interior of the mitochondria. On the other hand, the enzymes which catalyse protein synthesis are located on the microsomes. The cell, as a whole, is a true metabolic unit. We may mention here the recent discovery of factors in the chain of reactions which link the genes and enzymes. It is conceivable that in ten or twenty years it may become possible to furnish a biochemical explanation of the laws of heredity.*



Although numerous species have colonized dry land, many other plants and animals continue to lead an aquatic existence. On the left, a crustacean of the sub-class Copepoda, on the right a sea anemone (photo Lod).

Boundaries of living cells

Philippe SENGEL

We have seen that cellular protoplasm is characterised by its continuous chemical activity. But a cell cannot be separated from its environment, since exchanges of ions and molecules take place constantly. However, the internal chemical organisation of the cell remains to some extent independent of the environment. How is this independence maintained? How is it that the substance forming the cell should generally be quite different from that of the extra-cellular liquid? Here there arises the concept of a barrier separating the living intracellular substance from its inert surroundings. The cytoplasm is limited by a thin membrane, the plasma membrane, controlling the entry and exit of molecules and ions.

Structure of the plasma membrane

The plasma membrane's fine structure can only be seen in detail through an electron microscope. Through such a microscope it can be seen that the cellular surface differs in appearance according to whether it is in touch with other cells or free, immersed in an external substance (blood or lymph). Cells in contact with one another fill up the remaining space, which explains the mechanical solidity of epithelia.[1] The exposed surface is covered with minute prominences of a diameter of about 500 Å and measuring between 0·1 and 0·2μ in length; these microvillosities are limited by a very thin line of dense osmiophil substance,[2] resembling that of the fundamental substance of the cytoplasm.[3] The free surface of the cell can also form itself into a sort of depression which sinks into the cytoplasm. These structures have certainly got a functional meaning, since it is partly owing to them that the cell can incorporate molecules from the outside environment (figs. 3, 4 and 5).

The structure of the plasma membrane can be presumed to be very easily disturbed and unstable: it is continuously perforated and continuously reforms. Also the depressions of the membrane often connect with the endoplasmic reticulum.[4] The molecular structure is of a lipoproteic nature (fig. 2), as most probably is that of the membrane of the chondriosomes.

Permeability of the plasma membrane

The peculiarity of the plasma membrane whereby it permits exchanges between the cell and its environment is called permeability. This peculiarity is of vital importance for the maintenance of satisfactory physiological conditions within the cell: it is a selective function, that is to say, the membrane is permeable to some substances and not to others, admitting those which are useful to the functioning of

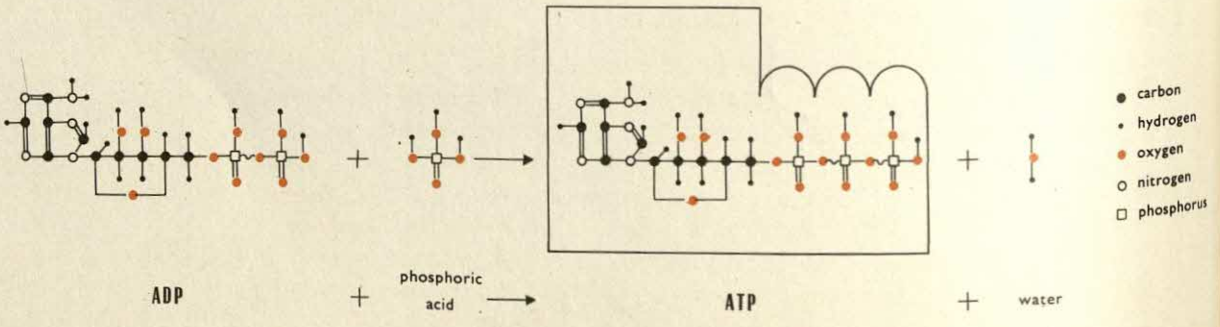


Fig. 1. Chemical structure of ADP *and* ATP. *Adenosine diphosphate* (ADP) *is combined with a molecule of phosphoric acid* (H_3PO_4) *to form adenosine triphosphate* (ATP) *and a molecule of water* (H_2O). *This reaction absorbs a great quantity of energy (about 8,000 cal.) which is thus stored in the phosphate links rich in energy (represented by a wavy line).*

(1) *On the cell in general and epithelia in particular, see the following chapter 'From cell to tissues'.*

(2) Osmiophilia: *See chapter 'The techniques of biological research'.*

(3) *In some cells (intestinal epithelium) the free surface takes the form of 'bristles' where the 'hairs' are only minute microvillosities, rather long and close together.*

(4) Endoplasmic reticulum: *See chapter 'Life-giving molecules'.*

the cell and rejecting those which would be harmful.

How do substances pass through the membrane? Many soluble bodies penetrate inside the cell because they can dissolve in the fatty layer of the plasma membrane.

However, the very fact that water and water-soluble substances can also break through the cytoplasmic barrier leads us to suppose that the fatty layer must be endowed with open pores so that such substances can pass through; it is also recognised that some chemical particles unite with a molecule of the membrane to form a compound able to migrate through it. Special enzymes, the permeases, play a part in this transfer process, catalyzing the compound and rebuilding the migratory composite on the other side.

At all events, in all these transfer processes, the plasma membrane shows remarkable selectivity: for example potassium and sodium ions penetrate quicker than ions of magnesium and calcium; nitrates penetrate quicker than chlorides, chlorides quicker than sulphates.

In some cases, the membrane not only selects but also plays an active part. Potassium, required for muscular contractions and in the conduction of nervous impulses can always be found in a much more concentrated form in the cell than in the extracellular liquid:[5] so it must therefore be assumed that potassium ions find their way into a cell not by simple diffusion but by a system of 'pumping' helped in this by special localised enzymes on the membrane. The energy required for the transport is supplied by energy-rich phosphates, eg adenosine triphosphate (ATP) (fig. 1).

The membrane is not always equally permeable; many factors have an influence on this function, such as the age of the cells, their physiological state, the temperature and nature of the external surroundings. Permeability can vary greatly, as can be seen by the following experiment: if animals are fed with a diet lacking in potassium, the muscle cells replace almost 50 per cent of their potassium ions with sodium ions, whereas normally these cells are virtually sodium-free (Heppel, 1939).

Importance of osmotic pressure

Since the cellular membrane is pervious to water and ions, it is essential that the cell be contained in an isotonic environment, that is, an environment where the osmotic pressure is the same or almost the same as that of the cytoplasm,[6] as shown by de Vries and Hamburger. The latter noted that the red corpuscles of mammals remained intact when plunged into a solution containing 0·951 per cent of sodium chloride. In a more diluted solution, on the contrary, these red corpuscles swell and the haemoglobin filters into the surrounding liquid: the swelling increases by the penetration of water, and the corpuscles finally burst. This is the phenomenon of haemolysis. In more concentrated solutions, the erythrocytes shrink through lack of water and become crinkled.

In plants, the intracellular osmotic pressure is always greater than that of the external environment, which maintains the cell in a state of turgescence and strengthens the soft parts of the plant (leaves). Plant cells have a second membrane, made of cellulose, which acts in a purely mechanical way in order to prevent cells bursting under osmotic pressure, as this can in fact reach up to 100 atmospheres.

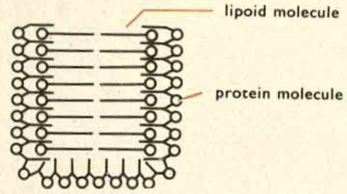


Fig. 2. Chemical structure of the cellular membrane.

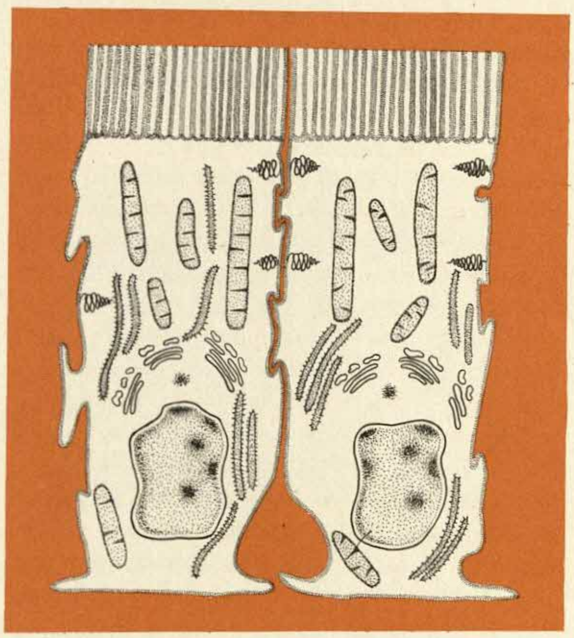


Fig. 3. Diagram of two cells in contact. The external surface is covered with microvillosities. The surfaces in contact show prominences that expand into the hollow of the neighbouring cell (from Bessis: 'Ultrastructure of the cell', Edition Sandoz, 1960).

Phagocytosis and pinocytosis

From the above we can deduce that the plasma membrane plays a vital part in the feeding of the cell. Most of the cells are fed by solutions passing through the membrane by the methods just described.

In some cases however the cells are able to digest actively solid or liquid particles of

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(5) *Muscle cells contain 300 mg of potassium ions per 100 gr of fresh tissue; red corpuscles contain up to 400 mg per 100 gr, whereas in the serum the concentration of potassium is about thirty times less.*

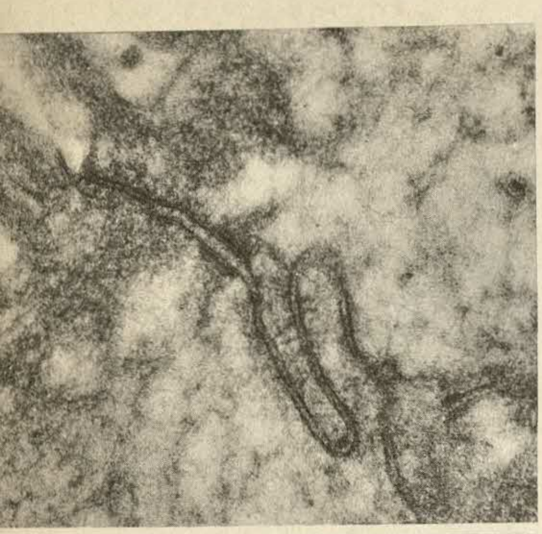
(6) *The study of the osmotic properties of the cell has led several biologists (Ringer, Locke, Tyrode, Knop) to prepare artificial liquids able to maintain life in cells outside the organism: these are physiological liquids or artificial sera.*

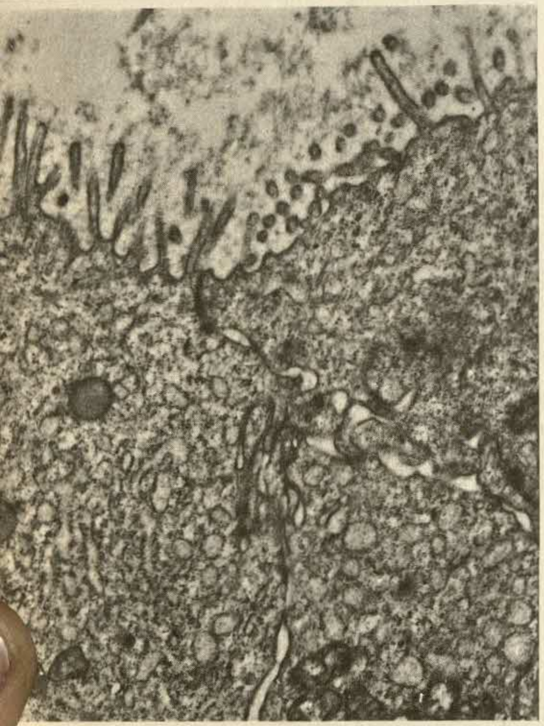
(7) Phagocytosis: *from the Greek word 'phagein', to eat. See chapter 'From cell to tissues'. Cells able to absorb in this way are called phagocytes or macrophages.*

(8) Pinocytosis: *from the Greek word 'pinein', to drink.*

(9) *For example, the disappearance of the tail of the larva of the tailless amphibia during metamorphosis, or the destruction of the red corpuscles in the spleen.*

(10) Rhopheocytosis: *from the Greek word 'rhophein', to absorb.*





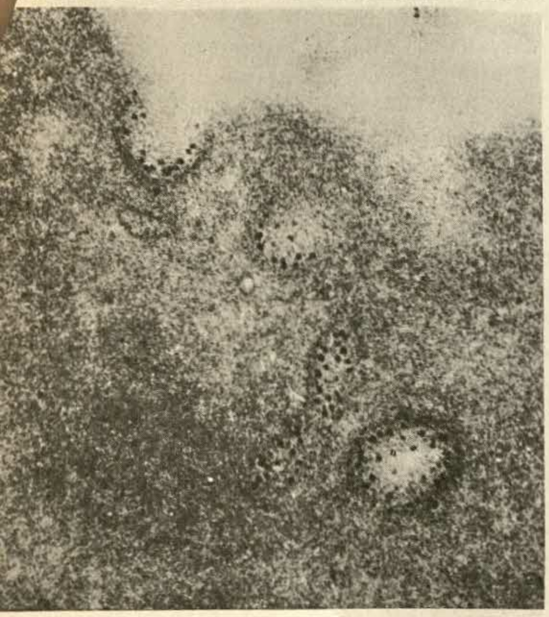


Fig. 4. Detail of the intracellular surface photographed by means of an electronic microscope (mag. × 75,000) (photo Dr Bessis).

Fig. 5. Detail of microvillosities which bristle up on the outside of the cellular membrane, photographed by means of an electron microscope (mag. × 12,500) (photo Dr Bessis).

Fig. 6. Rhopheocytosis is the phenomena whereby erythroblasts absorb iron molecules seen here as pinpoints (mag. × 225,000) (photo Dr Bessis).

such a size that the phenomenon is visible under an ordinary microscope. The ingestion of solid particles is called phagocytosis[7] and that of liquid droplets pinocytosis.[8]

Phagocytosis consists of two phases: first of all the particle to be ingested is adsorbed on to the surface of the cell by a process similar to agglutination, where physico-chemical factors play a part, such as the electrostatic load of the cellular surface.

In the second phase, the cell pushes forth cytoplasmic extensions which absorb the particle and finally swallow it into an intracytoplasmic vacuole. The plasma membrane then reforms by a linkage of the extensions.

Phagocytosis is a common occurrence in the protozoa, where it frequently forms the sole means of feeding; amoebae swallow large particles (micro-organisms such as bacteria for example) which they seize with their pseudopods and enclose in a digestive vacuole. In more developed animals, this phenomenon of phagocytosis is reserved for certain specialised cells, such as the leucocytes and macrophages of the reticular-endothelial system. Here phagocytosis is no longer a means of feeding but a defence system against the invasion of foreign particles, such as bacteria, dust, etc., or a means of recovering the constituent elements of cells which are degenerating.[9]

Far more wide-spread than phagocytosis, in the animal as well as in the plant world, pinocytosis was first observed in 1931 by Lewis studying cells cultivated *in vitro*. The manner in which droplets are absorbed does not differ greatly from that which applies to solids. The same cytoplasmic extensions can be seen, swallowing up the liquid vesicle and taking it into the cytoplasm in the form of small vacuoles. The size of these drops varies greatly; sometimes they are visible under an ordinary microscope, sometimes only an electronic microscope reveals them at all.

Recently Bessis has shown another method of swallowing up liquid droplets; he has called this phenomena rhopheocytosis.[10] Substances pass from one cell to another, as when the erythroblasts of the bone marrow absorb iron molecules which are supplied by a neighbouring reticular cell (fig. 6).

The membrane is essential to life

A membrane is much more than a simple boundary: it is in truth one of the most important elements of living matter. Without it, living matter would spread and be lost in the chaos of the inert world. It is within the membrane that the chemical reactions take place which characterise living protoplasm; it safeguards the structure as constant features show: thus most of the potassium and magnesium within a living being is restricted to the inside of the cell, whereas most of the sodium remains in the extracellular liquid.

Equally important is the fact that the membrane separates but does not isolate: through it, the cell is fed and unassimilated substances are rejected. It also plays a basic part in phenomena which are characteristic of life: sensitivity and mobility. It is at the level of the membrane that the cell is in touch with external stimuli and it is thanks to its flexibility and elasticity that the cell is able to move.

When a cell dies, the membrane loses its essential characteristics; it ceases to protect, to separate, to select the exchanges between the cell and its environment. Its functions are inextricably tied up with life itself.

Life-giving molecules

Roger and Colette VENDRELY

Nucleic acids were discovered about ninety years ago, but it is only during the last two decades that the importance of these substances has been fully appreciated. The number of research workers studying nucleic acids increases constantly, covering many fields of activities: biochemistry, cytochemistry,[1] electronic microscopy, virology, genetics and macromolecular physics. All the techniques of modern biology are directed towards these substances which have emerged during the past twenty years from the obscurity of poor relations to hold first place in the field of biological science.

At first only two kinds of nucleic acid were known: deoxyribonucleic acid (DNA), extracted principally from the thymus gland of the calf, from fish roe, the erythrocytes of birds, and ribonucleic acid (RNA) mostly obtained from beer ferment. It was also known (Kossel) that DNA was associated with a basic protein, histone, in various animal tissues, but that in the spermatozoa of some fish, this associated protein had an even more basic character, due to a high content of arginine: this was protamine.

A hydrolysis of nucleic acids showed that they were composed of phosphoric acid, sugar, purine and pyrimidine bases.

	DNA	RNA
	phosphoric acid	phosphoric acid
SUGAR	deoxyribose	ribose
PURINE BASES	adenine guanine	adenine guanine
PYRIMIDINE BASES	cytosine thymine	cytosine uracil

The two nucleic acids differ therefore in their sugar content: deoxyribose on the one hand and ribose on the other, and by one of the pyrimidines: thymine for DNA, uracil for RNA.

A less complete hydrolysis can separate from the nucleic acids nucleosides formed by the union of base and sugar, for example: thymidine = thymine + deoxyribose.

A more delicate hydrolysis separates more complex units: the nucleotides, formed by the union of a base, sugar and phosphoric acid, for example, thymidilic acid (thymine + deoxyribose + phosphoric acid). The molecule of nucleic acid was at that time considered as a collection of the four different nucleotides which are part of it, and Levene's tetranucleotidic formula was classical:

P—sugar - adenine
|
P—sugar - thymine
|
P—sugar - guanine
|
P sugar - cytosine

(P = phosphoric acid)

In this case, the molecular mass of DNA would have been about 1300. Actually it must be much higher.

The original mistake, when DNA was considered to be exclusive to animal cells and RNA to vegetable cells, was soon corrected; RNA was found in animal tissues and conversely DNA in vegetable cells. It was established that nucleic acids were divided not between the two main groups of living beings (animals and plants) but between the two parts of the living cell (the nucleus and the cytoplasm), DNA being restricted to the nucleus and RNA being more specifically characteristic of the cytoplasm.

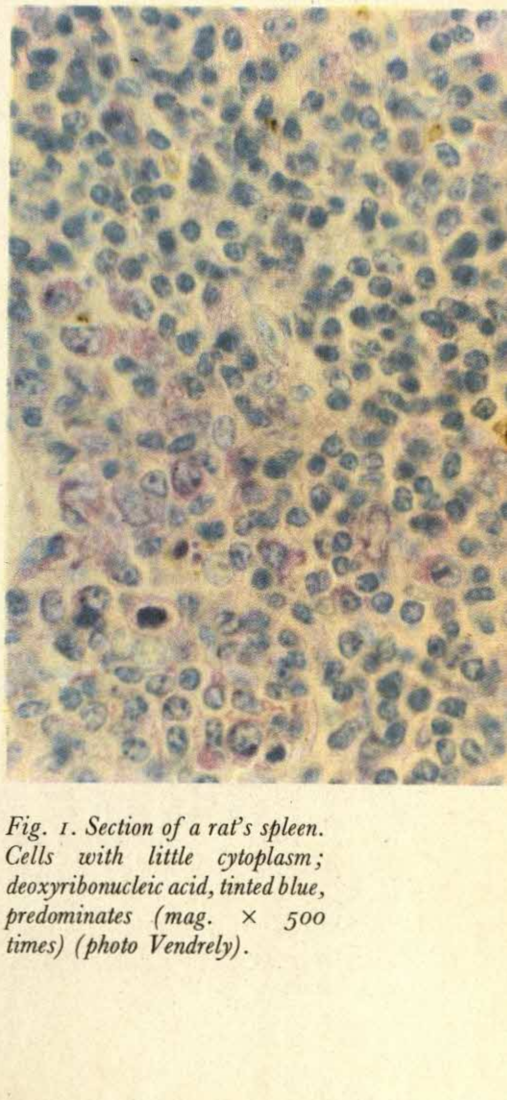


Fig. 1. Section of a rat's spleen. Cells with little cytoplasm; deoxyribonucleic acid, tinted blue, predominates (mag. × 500 times) (photo Vendrely).

The importance of these substances in the composition of living matter soon became evident, especially when Stanley and Bawden (1935–40) found for the first time that viruses, representing living matter in its more elementary form, could be formed solely by nucleo-proteins.

The real advance in the research into these nucleic acids dates from that time: the improvement in the methods of cytochemistry and histochemistry[1] made it possible to outline the theory of the part played by RNA in the synthesis of proteins,[2] a theory which has since been confirmed and elaborated and which has been the inspiration of many research projects.

Localization of nucleic acids in the cell

First of all, the actual position in the cell of the two nucleic acids was established: DNA is situated solely in the nucleus in the form of the chromatin; it is localised in the dark bands of the giant chromosomes of hironomus and rosophila larvae; that is, in the genetically active parts of the chromosomes. RNA, although mainly located in the cytoplasm, can also be found in the nucleus, especially at the level of the nucleolus.

The RNA content of the cell has been studied in many animal and vegetable tissues, and under many varying physiological conditions. These studies have brought forth the following fundamental ideas:

(1) RNA is very abundant in cells which actively synthesize proteins (for example in the active parts of the pancreas, in the cells of the stomach which secrete pepsin, in the liver, the salivary glands,[3] the neurons, oocytes, or glands of silkworms). Experimental procedures which seek to stimulate or slow up the synthesis of the proteins immediately influence the concentration of RNA in the cell. All the studies devoted to this subject prove that there is a *close correlation between the* RNA *content of a cell and its ability to synthesize proteins.*

(2) Embryonic cells and cells able to multiply rapidly (tumours) have a particularly high RNA content. This concentration of RNA diminishes when the embryonic cell is differentiated and acquires the characteristics which it will keep when adult. A cell which actively synthesizes proteins has a typical appearance which Caspersson has defined thus: a voluminous nucleolus rich in RNA, much RNA concentrated in the cytoplasm, especially touching the nuclear membrane.

Biochemists, in particular the followers of Davidson in England, have studied the nucleic acid content of various tissues and have confirmed the information given by cytochemists: cells carrying out active proteic synthesis are rich in RNA. The case of bacteria is particularly clear: Vendrely (1946) was able to prove that bacteria, in the same way as other cells, contained RNA and DNA, and that the RNA was particularly plentiful in cultures in the logarithmic growth phase, whereas in inert cultures it was less plentiful.

So, at first, it was RNA which was the centre of attraction and subject of investigations by cytochemists and biochemists alike, since it lent itself well to experiments because of the considerable variation shown during the various physiological and pathological states and experimentally produced conditions; and also an important factor was its probable role in the synthesis of proteins, one of the main functions of the living cell.

DNA—its role in heredity

DNA on the other hand is much more stable and not nearly so easy to experiment with as RNA. In 1944 a new fact came to light: Avery, McLeod and McCarthy proved that the transformation phenomenon in bacteria was due to DNA, which thus appeared, as Boivin expressed it, as the vector of hereditary characteristics and the chemical substratum of heredity.

What is this phenomenon of transformation?

In 1928 Griffith noticed that a particular strain of pneumococcus developed *in vitro* under special conditions lost its power of synthesizing the polysaccharide forming its capsule and from then on grew in a solid medium as rough colonies which had no pathogenic activity, whereas the virulent encapsuled cells formed smooth colonies. Griffith injected into a mouse a living pneumococcus culture with no capsule and dead pneumococci with capsules. The mouse died of septicaemia and he found in the corpse smooth, living pneumococci, showing precisely the same specificity as the killed germs he had injected.

Later it was shown that this transformation could be carried out *in vitro*, placing in the culture medium an extract prepared from these transformed cells. The transformation was permanent, the transformed germs did not revert to their original type.

Avery and his colleagues proved that the active principle of the transformation was DNA extracted from smooth encapsulated strains of pneumococci. The transformed germs reproduced indefinitely in the same manner and the DNA present showed the same transformation power. This DNA taken by the bacteria is therefore able to carry out its own duplication as well as to induce a hereditary property (synthesis of the capsule). *It therefore fulfils the two functions which characterise a gene.*

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(1) *Cytochemistry differs from histochemistry insofar as its aim is to define the positions of given chemicals in the various parts of the cell itself (nucleus, mitochondria, various granulations, etc.), whereas histochemistry is concerned not with cells but with tissues. Cytochemistry is necessarily carried out under powerful microscopes or even electron microscopes.*

(2) *The oldest method for the cytochemical study of* DNA *was the Feulgen method (1924), based on the fact that the products of a partial hydrolysis of* DNA *re-colour basic fushsin previously decolourised by sulphuric acid (Schiff's reaction). The method perfected by Caspersson is based on the fact that nucleic acids specifically absorb ultraviolet rays of a wave-length of about 2600 Å. This quantitative cytophotometrical method makes it possible to measure in sections of tissues or in isolated cells the concentration of nucleic acid at any given point. The method does not distinguish between* DNA *and* RNA *and must be completed by Feulgen's technique. Finally, the cytochemical method used by Brachet consists in staining a section of tissue with Unna's mixture (methyl-pyronine green); the methyl green stains the* DNA *and the pyronine is fixed by the* RNA. *A check on a sample section using ribonuclease shows the great precision of this reaction which enables a precise localisation to be made, but not a quantitative measurement of the two nucleic acids.*

(3) *(Fig. 3) The salivary glands of a mouse showing a cytoplasm rich in* RNA; *(Fig. 1) by contrast shows a mouse's spleen, a non-secreting organ and poor in* RNA.

Similar transformations were obtained shortly afterwards by Boivin and his colleagues in France from colon bacilli and by Alexander from *Haemophilus influenzae.* Hotchkiss carried out some remarkable work on the transformation of pneumococci sensitive to streptomycin into pneumococci resistant to streptomycin by the DNA of resistant germs, and he was able to analyse in detail this transformation phenomenon.

Several generations of bacteria are required before the new DNA can acquire the faculty of carrying out its own autoduplication but finally a cell doubles the DNA content and transmits it to two daughter cells; the duplication is then carried on definitively. It is possible to transfer several hereditary characteristics by means of a single preparation of DNA.

Attempts have been made, but without success, to obtain transformations of pneumococci by DNA derived from other sources. These heterologous DNA can however penetrate into pneumococci and, without provoking change themselves, prevent the cell from reacting with a normally transforming DNA. So they are sufficiently similar to pneumococcus DNA to be absorbed by the cells; once there, they block the possible action of a transforming agent that has been derived from the pneumococci.

So, as it has also been proved that bacteria contain nuclear substances and genetic equipment similar to those found in higher organisms, one can justifiably consider that DNA must represent the active part of the gene in all living beings. Recently, Professor Benoit and his colleagues succeeded in transforming some of the characteristics of Peking ducks by injecting DNA prepared from Kaki ducks. These transformations are permanent and are still found in each succeeding generation of ducks. So this phenomenon can be compared to the transformation phenomenon in bacteria. However, this example is unique and all attempts at transformation of mice, chicken and newts have so far failed.

The DNA content in the nucleus is constant

In any case, if it be true that DNA is the main constituent of genes, some consequences must follow: first of all the DNA content of the nucleus should be constant for a given species, as is the number of chromosomes.[5]

The fact that the DNA content of nuclei is in fact constant has been much used in various biological fields. DNA can be used as a yardstick to refer to a single cell all biochemical data measuring the differences between other biological substances during the various physiological and pathological states (Davidson and his followers).

Another important aspect of the study of the DNA content of the nucleus concerns the determination of this characteristic content in various animal or vegetable species.

The DNA content of the nuclei of mammals is always very similar. The same homogeneity is found in birds, though here the DNA content is about three times less than in mammals.

In other cases, fish for example, considerable variations occur from one species to another, suggesting that there may be polyploid sets of chromosomes in each nucleus. In amphibia, variations are quite considerable and cannot be referred to differences between the number of genes.

It is very interesting to note the comparison between DNA content in the nucleus and the chromosome formula in the same species. In some cases, the cytologist finds it difficult to decide whether a difference in the number of chromosomes found in related species is due to fragmentation or duplication of chromosomes leading to polyploids. The DNA content of the nucleus can determine this.

Schrader recently devoted an important article to this work, using the DNA constant as an instrument of taxonomy; in particular, he showed how in Hemiptera there can be big differences even in species closely allied.

Finally, it is interesting to note that bacteria have one hundred times less DNA than the nuclei of birds for example. As to viruses with DNA, their nucleic acid content is a hundred times less than that of bacteria. These figures are, of course, only approximate.

So the study of the DNA content of nuclei has proved fruitful and confirmed the idea that this substance could represent the active element of the gene. Study of the renewal of DNA by means of radioactive isotopes shows that this substance has great metabolic stability which prevents rapid renewal of RNA.

(4) *At this point a new method of studying the cell should be mentioned: the separation of isolated cellular particles by grinding the tissues and separating them out in a centrifuge. Claude and his school (1941) developed this method and succeeded in isolating from the liver tissue of a rat, cytoplasmic granules called microsomes containing a considerable amount of cytoplasmic* RNA:

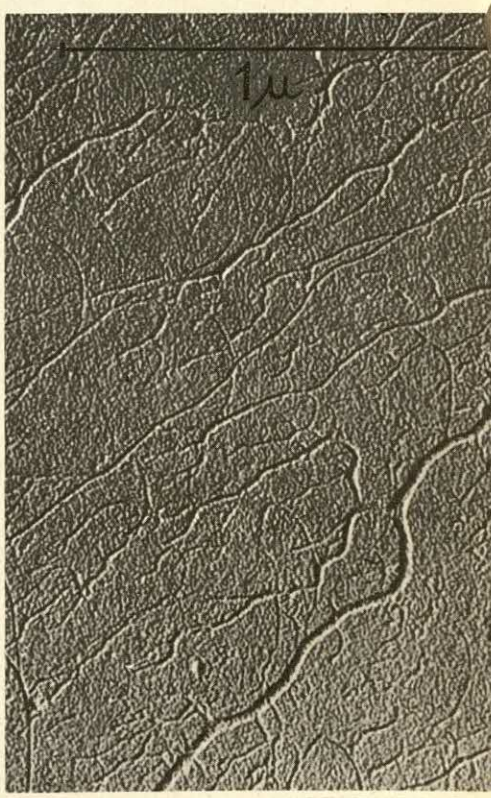

Fig. 2. DNA *of a calf's thymus gland seen under an electron microscope (mag. × 60,000 times) (photo Vendrely).*

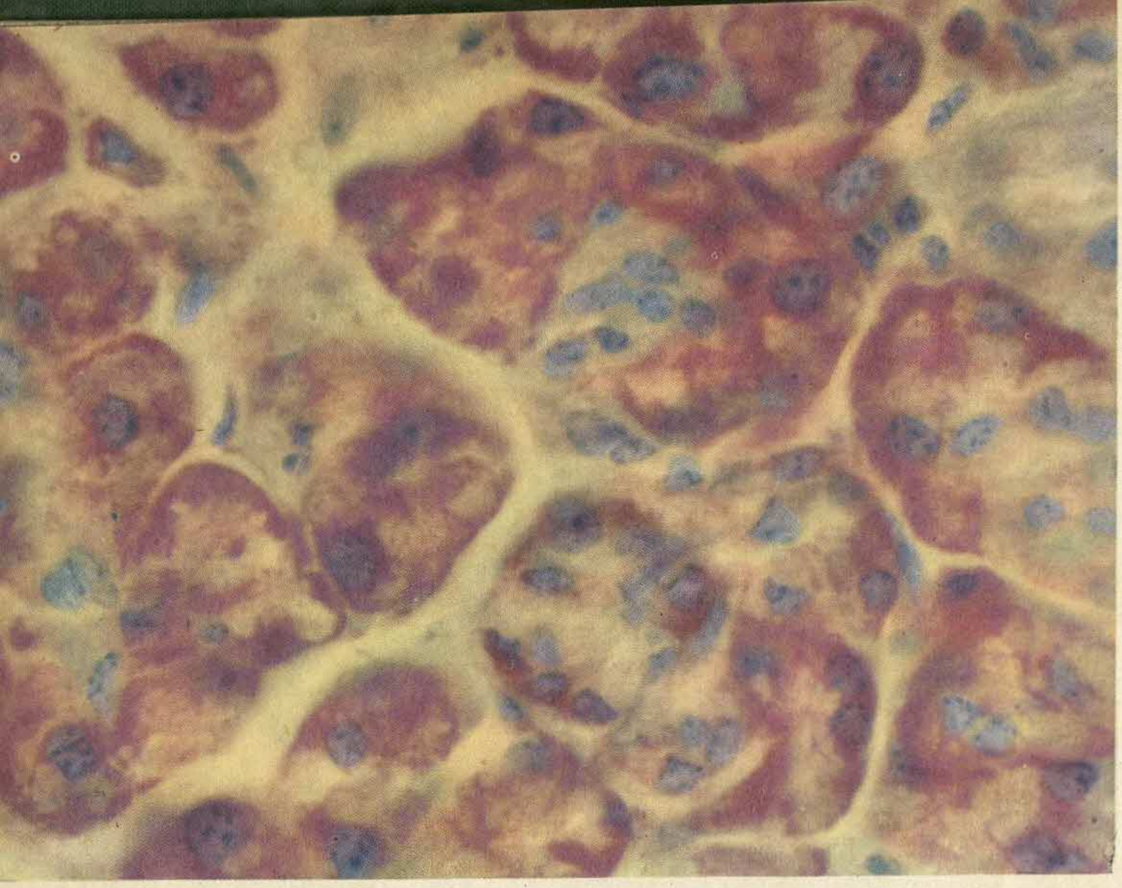

Fig. 3. Section of the salivary gland of a mouse, stained with methyl-pyronine green. The red colour reveals a high RNA *content in this tissue which synthesizes large quantities of proteins (mag. × 1500 times) (photo Vendrely).*

How can DNA contain the infinite variety of hereditary characteristics?

To have a genetic role it is not sufficient that DNA should be stable, it must also be able to bear in its physico-chemical structure the infinite variety of hereditary characteristics which appear in a living being. The DNA molecule, as shown by Levene with his tetranucleotidic structure, is obviously much too simple to fulfil this aim. A series of biochemical and physical studies have made it possible to learn more about this molecule and to establish a reliable theoretical structure for a substance endowed with such important properties.

From the moment when it became possible to prepare this substance in a non-degraded state, the physico-chemical and chemical study of the DNA molecule advanced greatly. The technique of Mirsky and Pollister (1946) is the one most used.

DNA thus isolated has been studied from the biochemical and physical points of view. The technique of chromatography used by Chargaff and his colleagues has made it possible to determine the proportion of mononucleotides of each kind to be found in a DNA molecule. Samples of different origin have been studied: Chargaff studied DNA derived from different tissues of the same animal species and from many different animal and vegetable species: mammals, fish, Echinodermata, plants, micro-organisms, viruses.

Results obtained have made it possible first of all to refute finally the hypothesis of the tetranucleotidic structure put forward by Levene. In DNA, the proportion between adenine and guanine, thymine and cytosine, for example, is very rarely equal to one. Animals and higher plants have more adenine than guanine, more thymine than cytosine.

The proportion between the bases also differs from one species to another: significant statistical differences exist, even for species not too far apart, such as oxen and man. The differences are much greater in micro-organisms than in higher forms.

DNA from various organs of the same animal or from various strains of a single bacterial species have approximately equal proportions between the bases. In all the DNA preparations examined up till now the ratio total purines/total pyrimidines is always near to one, in the same way as the ratio adenine/thymine and guanine/cytosine is also nearly equal to one.

It would seem therefore that *there is an obligatory balance between purines and pyrimidines;* the proportion of a purine base, adenine for example, imposes a similar proportion of a pyrimidine base, thymine. So the bases could therefore be linked as follows: adenine/thymine, guanine/cytosine. This precise and positive fact has been usefully employed in setting up the schematic diagram for DNA.

It has been found also that there are two main types of DNA, the AT type, where adenine and thymine are predominant, and the GC type, where guanine and cytosine are predominant. This latter type has only been found in micro-organisms, where an intermediate type is also found.

As well as these biochemical facts, other valuable information has been obtained by physical and physico-chemical methods. The electrotitration measurements have shown that the CO and the NH_2 groups of bases cannot be accessible to titration, even in an aqueous solution, whereas the phosphate groups are fully accessible. This necessarily implies that, in the molecule, the basic groups are concealed either by their central position or by a bond or for both reasons.

Physico-chemical methods of sedimentation, diffusion, and diffraction of light, have given definite information regarding the form and size of DNA fibres. These fibres are rigid, about 20 Å units in diameter and several

(5) *Boivin and his collaborators, Roger and Collette Vendrely, studied the* DNA *content of the nuclei of various tissues of bovines and spermatozoa of the same species. They established that the* DNA *content of the nuclei was remarkably constant in the various organs of any single animal and that the spermatozoon contained only half the* DNA *content of the somatic nuclei. These facts have been confirmed and reinforced by the use of the cytophotometric method on visible preparations by Pollister, which enables the* DNA *content of the nuclei to be measured in histological sections after being coloured by the Feulgen method. Today it seems certain that the* DNA *content of the nuclei is connected with the number of chromosomes. It is doubled in those cells about to divide and it is multiplied by 2, 4 or 8 in the tetraploid, octoploid or hexadecaploid cells.*

hundred Å in length (fig. 2).

Finally, the examination of the x-ray diffraction diagrams of DNA fibres (Astbury, Crick, Wilkins and Miss Franklin) has made it possible to establish that nucleotides must be set perpendicularly to the axis of the fibre and arranged one above the other, about 3·4 Å apart. The 'period', according to the axis of the fibre, that is the distance at which an identical lay-out of atoms occurs, is 34 Å.

Helicoidal structure of the DNA molecule

The idea of a probable helicoidal structure of the DNA fibre was put forward after study of the x-ray pattern, the axis being parallel to the length of the fibre.

All these various facts and data make it possible to conceive of a model of the molecular structure of DNA which seems to be satisfactory so far as our knowledge goes at present. The simplified figure (fig. 4) shows the Watson and Crick model. The DNA molecule is represented by two polynucleotide chains linked together and wrapped helicoidally round a common axis, the two chains being held together by hydrogen bonds between purine and pyrimidine bases. The linked bases are placed perpendicularly to the axis of the fibre and so are the glucoside bonds between sugars and bases. The plane of the deoxyribose rings is parallel to the axis; each chain completes one complete revolution every 34 Å; phosphorus atoms are placed 10 Å from the axis, so that the total diameter of the fibre is 20 Å.

Watson and Crick placed the phosphate groups on the outside of the helix, and the bases on the inside; these can be bonded by hydrogen, which is in conformity with the electro-titration information. As the nucleotides are placed one above the other, hydrogen bonds between bases cannot occur along the same chain. It is therefore bases from different chains which must be linked together. To maintain a perfect helicoidal shape and to have the chains always equidistant, it is obligatory each time to link one purine base with one pyrimidine base. Bases can only be linked by specific pairs: adenine bonding to thymine and guanine to cytosine.

This diagram is very attractive because it represents the integration of almost all information so far obtained on the structure of DNA.

This model also explains how a DNA molecule can undergo reduplication. Watson and Crick conceived of a process of separation in which the two strands of the double chain opened like a zip fastener. On the bases thus exposed, specific nucleotides held in reserve in the cell would fix themselves: a nucleotide adenine would fix itself by its base to the base of a thymine nucleotide fitted in the chain. The new series of nucleotides thus placed parallel to the template chain could finally be polymerised into a long new chain under the action of a specific enzyme.

The Watson and Crick model shows the immense possibilities of variations in the sequence of nucleotides along the DNA chain. Gamow has mentioned that in a chain of 100 nucleotides of the four kinds (and most DNA chains are probably even longer), the possible permutations can number 4^{100}, that is, a number 100 times greater than the total number of atoms in the solar system. If, as seems probable, the genetic information is expressed in the order in which the nucleotides appear, there are quite enough permutations to account for all the genes of chromosomes.

How is genetic information transmitted?

Thus the DNA molecule by its structure and complexity corresponds very well to the idea of a carrier-molecule with hereditary factors or genes; but how is this genetic information transmitted to the protoplasm? We do not know. Caspersson's work suggested a process originating in the chromosome and leading to the synthesis of the cytoplasmic proteins; the nucleolus would serve as a relay between the chromosome and the RNA of the cytoplasm which in turn would directly control the synthesis of the cytoplasmic proteins.

Although the nucleus is essential to maintain cytoplasmic RNA over a long period, protein synthesis in the cytoplasm can easily be carried out without a nucleus, as was shown by Brachet in enucleated fragments of *Acetabularia*. An enucleated fragment of *Acetabularia* continues to synthesize proteins thanks to the microsomes it contains, but the presence of the nucleus is essential for the renewal of these microsomes.

Palade, using electronic microscopy, proved that in an intact cell microsomes are represented by a system of vesicles or tubes called the 'endoplasmic reticulum' which connect together and are surrounded by a membrane having on the outer surface granules with a diameter from 100 to 200 Å. These granules, called Palade's granules, are

(6) *Here also must be mentioned the remarkable progress made recently in biosynthetic knowledge of nucleic acids: the authors were awarded the Nobel Prize for this work. In 1955 Ochoa and his collaborators isolated from a micro-organism* —Azobacter vinelandu—*an enzyme they named polynucleotide phosphorylase; this enzyme acts as catalyst in the synthesis of ribopolynucleotides highly polymerised from nucleotides 5, diphosphates. These polynucleotides are hydrolysed by ribonuclease, phosphodiesterase and alcali exactly in the same way as* RNA. *They have even been combined with the protein of the tobacco mosaic virus to form particles which resemble non-infectious viruses.*

Kornberg and his colleagues (1958–9) succeeded in preparing from Escherichia coli *a purified extract containing an enzymatic system still ill-defined which, in the presence of a mixture of*

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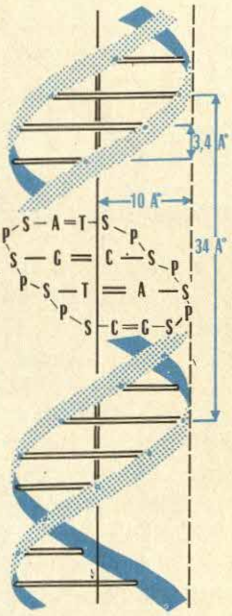


Fig. 4. Helicoidal structure of DNA. *Schematic diagram conceived by Watson and Crick. The vertical line shows the axis of the fibre. The two ribbons represent the two chains made up of sugar and phosphorus groups. The horizontal lines represent the pairs of bases holding the two chains together (photo Vendrely).*

Continued from previous page

deoxynucleotide and triphosphates of adenine, guanine, cytosine and thymine and a small amount of DNA *acting as primer, produced a polymer very similar to* DNA. *The nucleotides of the chain would be connected by phosphodiester bonds, 3, 5, like the natural product and the molecular weight of the polymer would be about* 5.10^6. *To be noted: for this synthesis to be possible, all four nucleotides must be present simultaneously.*

macromolecules of ribonucleoproteins. They can be isolated if the microsomes are treated with deoxycholate which dissolves the membranes.

There is every reason to believe that it is the RNA in these particles which forms the template on which the protein chains are moulded. Research by many scientists such as Hoagland, Zamecnick and his collaborators, on the incorporation of marked amino acids by the microsomes of a rat's liver have shown that particles of ribonucleoproteins are the seat of an initial incorporation of free amino acids. This process begins by activating the amino acid by means of an enzyme which then combines with the ATP and gives an amino-acylmonophosphate.

Zamecnick concludes that these constituents stretch along a ribonucleoprotein model used as the 'pattern', the order of the amino acids being determined by their ability to adjust to the particular form of the nucleoprotein surface; then these amino acids would connect together to form a polypeptide chain.

Hoagland has shown that activated amino acids are probably attached at first to soluble RNA of light molecular weight, which plays the part of carrier before the adsorption on the microsomic particles. The cytoplasm would therefore contain two kinds of RNA: one soluble and having a metabolic function, the other, represented by microsomes and used as a template for protein synthesis.

The study of plant viruses having no DNA has shown that in such cases RNA is the sole carrier of hereditary information: Fraenkel-Conrat and Williams (1955) proved this successfully on the tobacco mosaic virus, where particles are formed by a thick cylinder of protein and a central ribonucleic portion. They succeeded in separating and then reuniting the two components, RNA and protein, thus reforming an active virus. Further, they were able to prepare a hybrid virus, a combination of RNA from the plantain strain of the tobacco mosaic virus with protein from the ordinary strain. The hybrid virus thus obtained produced the characteristic disease of the plantain strain, but resembled the ordinary strain in its immunological properties. However, the protein of the descendants of the hybrid virus were identical to those of the plantain strain from which the RNA had been extracted.

So RNA *is capable of transmitting an obvious genetic message, since it can reform a protein similar to that of the original strain.*

Finally, Gale and Folkes working on the protein metabolism of staphylococci disintegrated by ultrasonic means, using marked amino acids, showed conclusively that nucleic acids play a part both in the synthesis of proteins and in the incorporation of marked amino acids in pre-existent proteins. These authors consider that the DNA organises the synthesis of the RNA according to a special template. RNA, formed under the influence of DNA, acts as a catalyst to the synthesis of the new protein at a speed which varies with its concentration.

So the part played by RNA in the formation of specific proteins becomes clearer; that this model is itself formed according to the genetic information contained within the DNA of the nucleus is certain, but how this information is transmitted still escapes us.[6]

The considerable development of research on nucleic acids has made it possible to take a definite step along the path of one of the most mysterious phenomena of life, to contemplate the synthesis of living matter, a synthesis strictly regulated by set plans transmitted generation after generation by the reproduction of these precious molecules which contain within themselves the hereditary message. Almost everywhere in the living world deoxyribonucleic acid is the carrier of this message, transmitted to the cell by means of ribonucleic acid which determines the formation of specific proteins controlling not only life in general but the very existence of each and every human being.

HISTORICAL NOTES

1868 to 1937
Discovery of nucleoproteins by MIESCHER in 1868, and the isolation of these substances from the tissues of many separate animal species (MATHEWS, PLASZ, ACKERMANN, LILIENFELD). Definition and preparation of nucleic acid (ALTMANN, 1889). Biochemical study and classification of many nucleoproteins (KOSSEL and his school, 1928). Work on the chemical structure of nucleic acids (KOSSEL, STEUDEL, JONES, LEVENE, HAMMERSTEIN, BREDERECK, GULLAND).

1935–40
STANLEY and BAWDEN established that viruses may be formed solely of nucleoproteins.

1937–46
Development of cytochemical research in the study of nucleic acids in tissues. There were more results relating to RNA *than any other; biochemical research confirmed and clarified the brilliant results obtained by cytochemistry. Histochemical study by the Caspersson school in Sweden and by Brachet in Belgium. Refinement of extraction methods (Mirsky and Pollister) and of quantitative study of the nucleic acids (Schneider, Schmidt and Tannhauser), which furthered biochemical studies (Davidson and his school); this confirmed and developed the facts established by Caspersson and Brachet.*

1946–55
Period during which considerable developments occurred in the study of DNA, *characterised by the attraction of physico-chemists and physicists, to the field, who add their knowledge to that of biochemists and cytologists.*

1953–60
Elucidation of the structure of DNA *by WILKINS, WATSON, and CRICK.*

Only the cell lives; everything else is merely an accessory, or, to express it in another way, is nothing but a mechanism so far as the cell is concerned.
CLAUDE BERNARD

PART TWO

From cell to organised being

All higher living creatures are made up of an aggregate of basic units and the basic structural units of living matter are the cells. *These might be called 'biological atoms' (atoms means 'indivisible'), for the biological properties of a tissue are retained only when at least one cell remains. The external shape and the internal composition of living things result from the arrangement of their cells and from substances produced by cellular activity. In the same way, the functional manifestations of live organisms results from the individual activity of their cells. Each cell individually carries on all the essential and characteristic functions of life: nutrition, digestion, respiration, motility, reproduction, etc. It is thus a morphological and functional entity enjoying a certain degree of autonomy, although it must be emphasised that its true value and significance are only acquired by virtue of the place which it occupies in the whole organism and by its relations with neighbouring cells.*

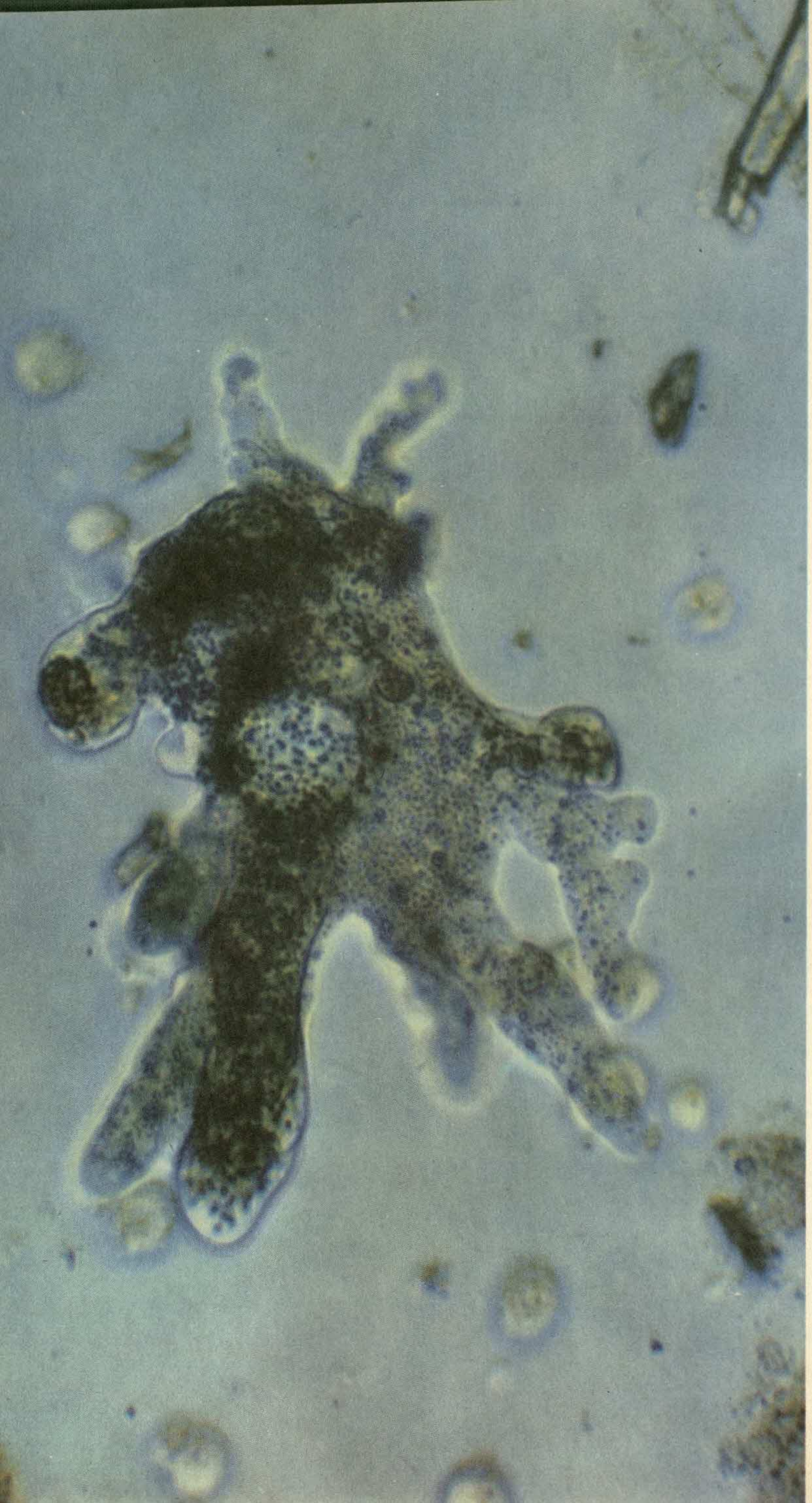
Very early in animal evolution, when unicellular organisms developed into multicellular ones, some cells became specialised to perform specific functions in addition to those necessary to their own survival. Later, such specialised cells became grouped into 'tissues': epithelial and glandular tissue, connective (supporting) tissue, cartilaginous and bony tissues, muscular and nervous tissue and blood (which may be regarded as a fluid tissue). Thus, a tissue may be defined as an aggregate of cells, of one or several kinds, arranged in a particular way and carrying out specific functions; it is classified by both its structure and its function.

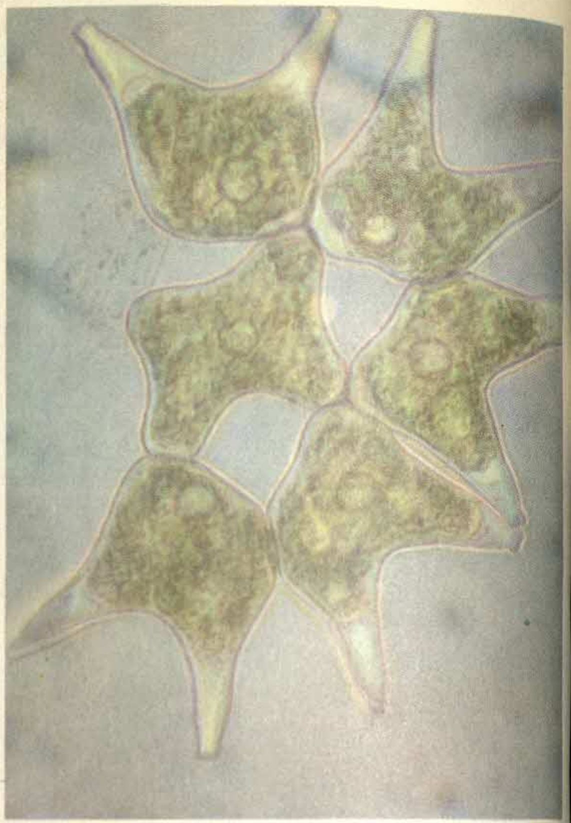
In general, the tissues of an organism exist separately but are found closely associated and intermingled, combining to form organs. An organ is a complex of tissues, one of which predominates and decides its principal function.

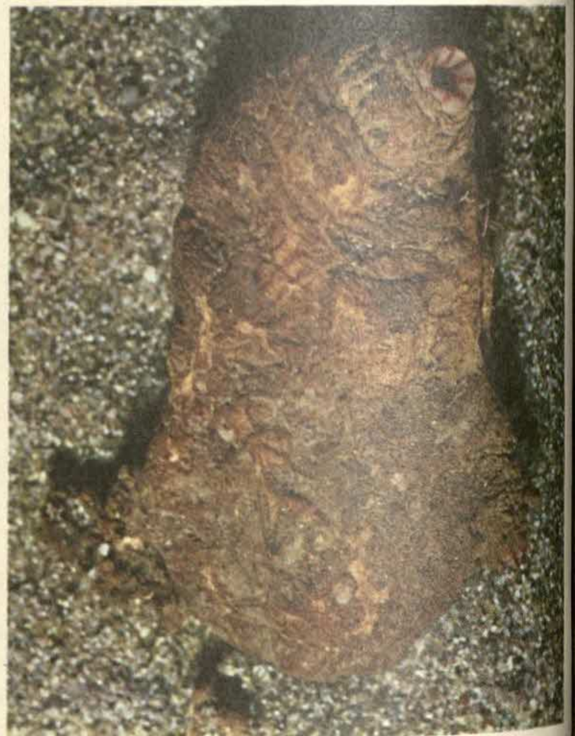
An attempt to study the fundamental properties of living matter by examining complex organs, would be as unrewarding as trying to study the structure of inorganic matter by considering, for example, a complicated machine tool. First, an analysis of the structure and behaviour of the smallest component units, the cells, must be undertaken and this we must attempt to outline.

The cell itself has an organised structure, the most important aspect of which is its subdivision into two parts, the nucleus and the cytoplasm, an arrangement which is found even in unicellular organisms and bacteria. Both parts are necessary and neither can exist on its own, for if isolated it degenerates after a very brief time. The nucleus is a central body separated from the cytoplasm by a membrane and containing special substances of prime importance, the nucleic acids. The cytoplasm is composed of a basic matrix with inclusions of various kinds. Although apparently structureless under the ordinary microscope, it is surrounded by a submicroscopic membrane. The inclusions may be constant or variable.

With rare exceptions, the cells of vertebrates are microscopic in size. However, when plentiful reserve materials accumulate in the cell, it may reach considerable dimensions, as in the case of the hen's egg, where the cytoplasm of a single cell has assumed enormous proportions, although the nucleus remains microscopic.

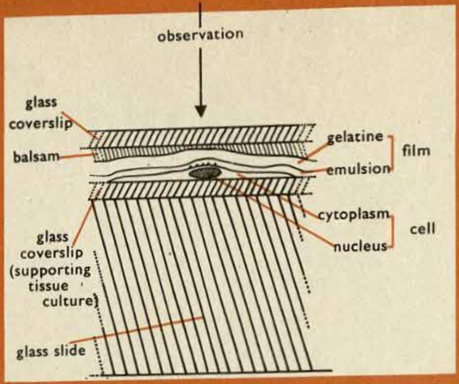






The amœba (left) is a unicellular animal. But higher up the evolutionary scale we find an increasing tendency for the cells to group themselves into tissues or organs. The Protococcus *(green alga) shown above is merely an assemblage of cells; the ascidian (sea-squirt), beneath is already an organised being with a mouth and a digestive tube; as for the dragon-fly on the right, it is an extremely complex creature with highly specialised cells (photo Lod).*

Fig. 1. (a) Diagrammatic drawing of part of a histoautoradiographic preparation (with tissue culture) cut perpendicularly to the surface. The relative proportions between the elements have been ignored here. (b) From left to right, four stages of mitosis in a histoautoradiographic preparation (see note 8); culture of chicken fibroblasts to which has been added a 'labelled' precursor of DNA *(thymidine marked with tritium); where there was* DNA, *silver granules have developed in the underlying photographic emulsion (mag. × 1000).*



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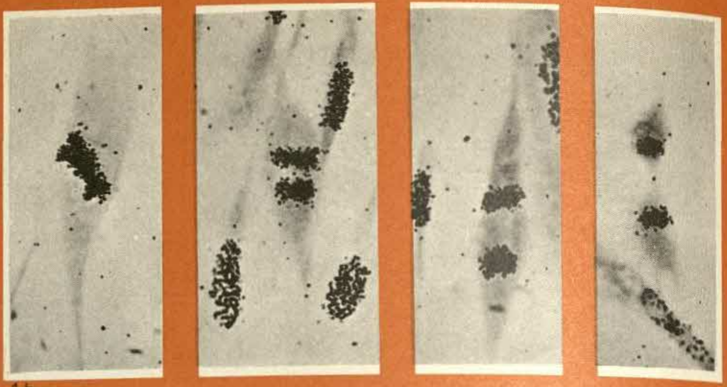

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Fig. 2. Emigration area of a culture of skeletal muscle and connective tissue (fixed and coloured) two days after explantation; on the left (in black) portion of the original explant (mag. × 30).

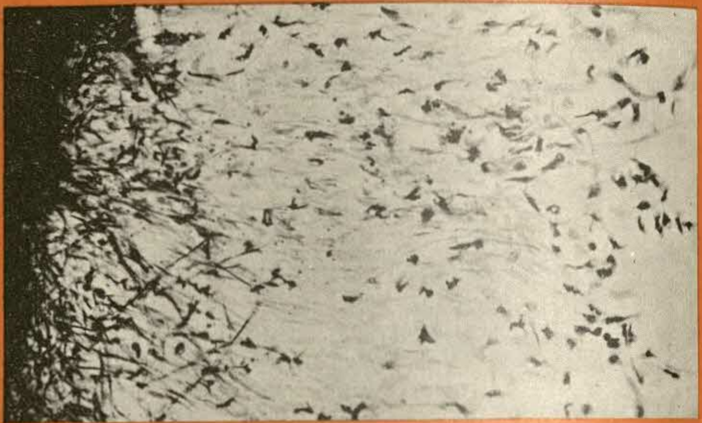

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Fig. 3. View of part of a live chicken fibroblast greatly enlarged (negative phase contrast); a certain structure of the cytoplasm appears at the limits of visibility (mag. × 3500); note also the Golgi bodies, the small grey area near the nucleus.

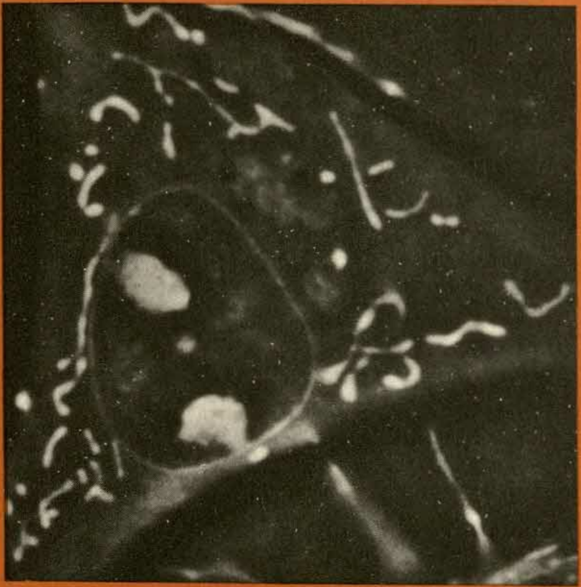

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Fig. 4. General view of skeletal muscles and connective tissue of a chicken fixed and stained, three days after explantation; in the centre: explant from which many cells have emigrated and multiplied. The fine black lines are muscular buds (mag. × 18).

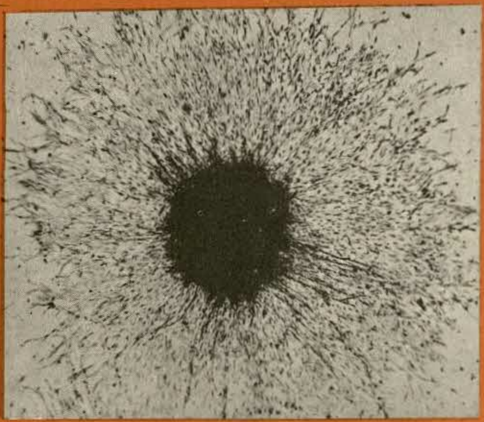

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Fig. 5. Successive images of the same part of a fibroblast (taken from a film in phase contrast); one minute between each picture; note change in shape of chondriosomes (mag. × 2400).

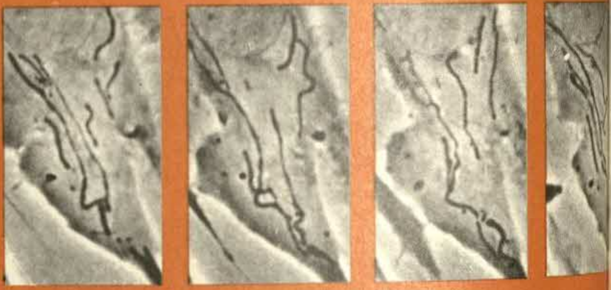

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Fig. 6. Change in shape of filiform chondriosomes; drawings made direct from analyses of films.

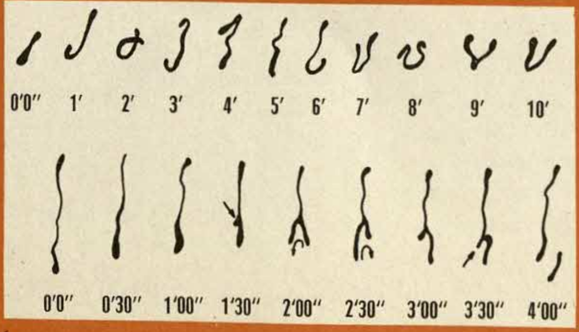

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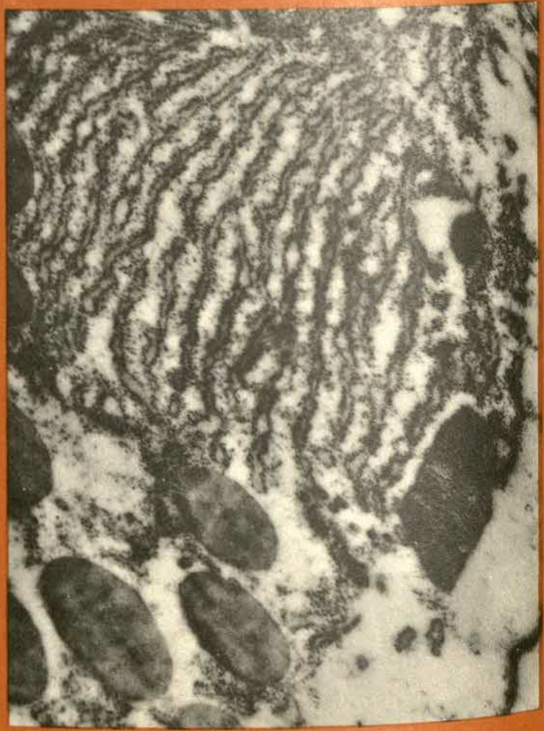
Fig. 7. Electronic photomicrography. Section of a glandular cell of the pancreas, after fixing and ultra-fine sectioning (0·02μ); top right, secretion granules; to the left a chondriosome (with internal lamellated structure); the fundamental substance of the cytoplasm is filled by a system of double membranes and small granules (mag. × 34,500) (photo A. Claude).



7

From cell to tissues

Maurice CHÈVREMONT and Jacques FREDERIC

The size of a cell varies from one tissue to another. However, apart from the very large nerve cells, most cells have a volume of between $200\mu^3$ and $4{,}000\mu^3$ (μ—micron—1/1,000 mm). A blood cell, for instance, has a diameter of 7μ and an intestinal cell is some 35–40μ long. The slender processes of some nerve cells, the nerve fibres, may be up to a yard long, while the cell body itself measures only about 100μ. The size of the cells bears no direct relation to the size of the individual they belong to: the cells of a dwarf are no smaller than those of a giant, merely less numerous.

Since cell size generally varies very little, the different weights of multicellular animal species depend chiefly on the number of cells present, the number being usually astronomical. Thus in man there are in the region of 1,000 million million cells of which 10 thousand million are nerve cells in the cerebral cortex, 25 million million red blood cells and 30 thousand million white blood cells.

The shape of a cell depends on its particular type and function, as well as mechanical pressures brought about by adjacent cells or tissues. For example, cells arranged in layers above each other, like the epithelial cells, become cubical or column-shaped; cells which are separated from one another, like the connective tissue cells, become elongated or spindle shaped; while those living in a fluid environment, like the red blood cells, tend to become rounded.

Living cells and tissues may be studied under the microscope with or without vital staining,[1] which is a method of staining cells with non-poisonous dyes. However, such cells soon degenerate, so dead or 'fixed' preparations are often used for microscopic examination. The specimen is cut into very thin sections by means of a microtome, which is a special device for doing this, and then stained by various techniques.

Fixing cells consists in treating them with physical and chemical agents which can preserve their structure as far as possible, and so prevent bacteria and their own enzymes from destroying them. Chemical agents are usually used, such as formalin, alcohol, picric acid and potassium bichromate. Fixing is a useful prelude to histological staining by acting as a mordant to absorb some of the stains used. The aim of this type of staining is to make the various structures of the material visibly distinguishable from each other, which is possible because different parts of fixed material vary in their ability to take up stains. Thus, it is easy to show up the nuclei, cytoplasm, connective fibres and cellular inclusions. The colour of the stain is immaterial since it tells one nothing of the chemical nature of the stained structure and may vary according to the technique used.

In this way histological staining differs fundamentally from histochemical[2] studies, which sets out to find out something about the chemical composition of the cell contents.[3]

However, the functions of living cells cannot be studied by looking at dead or dying cells, even under the highest magnification. Certainly, valuable information about the structure, composition and arrangement of the cellular components may be gained, but nothing about their behaviour. Therefore all morphological studies must also take into consideration the fourth dimension, time. Besides, as Carrell pointed out, each type of cell is characterised as much by its biological or functional properties as its morphological appearance.

From this point of view, tissue culture is particularly interesting. A small piece of tissue

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(1) Vital stain: *Cellular components, difficult to see or themselves invisible, can be shown* in vivo *as a result of the affinity they possess for certain colouring substances. A vital stain is a not very toxic substance which can be injected into the animal or placed in contact with a tissue culture or isolated cells (white corpuscles, for example). A fragment of tissue just taken from an animal can also be plunged into an isotonic solution of vital stain. According to requirements, granules, intracytoplasmic vacuoles, chondriosomes, the chromatin of the nucleus, etc., are selectively stained. The vital stain also serves to pick out certain cellular types, such as the macrophages (Fig. 18) or to show up special cellular activities such as the resorption by the cells of the renal tubes (Fig. 19). If some vital stains are well tolerated by the cell, others, unfortunately, are toxic and the cell can only survive for a short time. Amongst the vital stains in common use are trypan blue, isamin blue, Janus green, neutral red, etc.*

(2) Histochemistry: *some substances or chemical functions can be demonstrated in a tissue or cell by special chemical reactions. These reactions, which generally give a coloured product, are often very localised and make possible definite intracellular localisation. Ions, enzymes, organic substances, etc., can be demonstrated in this way.*

All the microphotographs in black and white and in colour throughout this chapter are the copyright of the authors, except figure 7.

(3) Cytophotometry: *When the results of a histochemical reaction is a coloured product and when the coloration obtained is proportional to the content of the substance shown, a quantitative evaluation is possible. In principle, it is sufficient to measure the absorption by the preparation of a stable luminous beam with a suitable wave-length (cytophotometry in visible light). The microscope is illuminated by monochromatic light and the image of the object to be measured, coming from this microscope, is projected through a small hole on the cathode of a photomultiplier, where the current produced is measured after being amplified. The Feulgen reaction for deoxyribonucleic acids lends itself particularly well to such research (Fig. 8).*

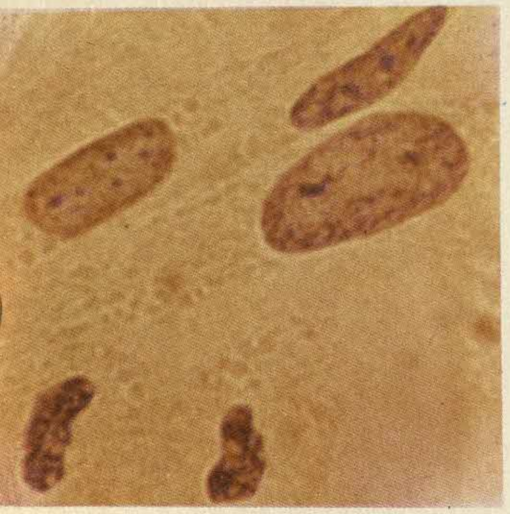

Fig. 8. Feulgen histochemical reaction to show deoxyribonucleic acids (DNA) *in a fibroblast culture of chicken. The Feulgen reaction leads to the formation of a mauve-red stain exactly where the* DNA *lies in the nuclei or chromosomes; there is no* DNA *in the cytoplasm which remains colourless. Such preparations lend themselves particularly well to cytophotometry (mag. × 1330).*

may be aseptically removed from a living organism and placed in a suitable medium *in vitro*, together with the necessary nutrients. This is what Carrell and Ebeling[4] did in 1912 when they cultured pieces of chicken heart. These experiments showed that as long as tissues isolated from an animal are suitably nourished and cared for, they are 'immortal' and are constantly renovated by the division of their parent cells.

A small piece of isolated tissue is usually cultured on a medium composed of blood plasma and various concentrations of embryonic extract. It may either be inverted under a thin slip of glass, in the technique known as 'hanging drop culture', or placed in various kinds of special flasks. The tissue is periodically sub-cultured onto fresh medium, thus ensuring that waste products are removed and more growth substances are supplied. Naturally, the cultures are kept at the same temperature as the animal they came from, which for chicken tissues is 37–39°C.

Amongst later techniques is culture in 'roller tubes' or on perforated cellophane membranes. The first consists in cultivating the tissue in tubes slowly rotating, whilst the second employs perforated cellophane as a solid substrate. The latter enables very large quantities of living cells to be grown—up to one pound. The use of synthetic media makes it possible to investigate which substances are essential for growth.

Tissue culture presents many opportunities for experiment. According to the technique employed we may cultivate rapidly-dividing cells, or slow-growing ones carrying out specific activities. Either entire organs may be cultured, or strains of normal or pathological cells which have been derived from a single isolated unit. The tissues cultivated *in vitro* being free from the effects of the blood stream, the nervous system, and hormonal control their behaviour can be studied with, or without, the addition of known external factors such as chemicals which modify cell metabolism, or hormones, or inhibitors of growth. Eventually, however, the cells become adapted to their mode of life *in vitro* and lose some of their typical characteristics.

Moreover, tissue culture makes it possible to prolong the direct observation of living cells growing either spontaneously or after experimental interference. This may be done under these circumstances in a particularly precise and detailed way by using the phase contrast microscope, microphotography and microcinematography.[5, 6]

Elements in perpetual movement

As an example, a culture of chicken connective tissue, like those shown fixed and stained in Figs. 2 and 4, may be examined alive under a phase-contrast microscope. Star-shaped fibroblasts can be seen; the different parts of the cells become visible—the cytoplasm, nucleus and nucleolus, the greyish filaments or rods known as mitochondria, as well as small black blobs and larger lighter ones, which are inclusions of water or fat.

The development of one of these fibroblasts can be followed by making drawings or photographs at intervals, or better still by using speeded-up ciné film. The cell is not immobile, but slowly changes its shape and moves about. Neither are its component parts immobile, for the mitochondria change position continually; the shape of the nucleus varies and the nucleoli move about and change. Apart from the movements of the mitochondria, these changes cannot be seen directly, except by means of comparisons of drawings or photographs. But, even when the cell is apparently completely motionless, it is in a state of intense activity at the molecular level, inaccessible to direct investigation. Thus the fundamental characteristic of life, motility occurs at all levels of organisation.

The contents of cytoplasm

The mitochondria (Figs. 3, 5 and 6) are very important cell components which are present throughout the life of both animals and plants. They may be studied either in cells that have been fixed and stained by special methods; in living cells examined by phase contrast (Figs. 3 and 5); or with an ordinary microscope after vital staining. It is also possible to examine their ultra-microscopic

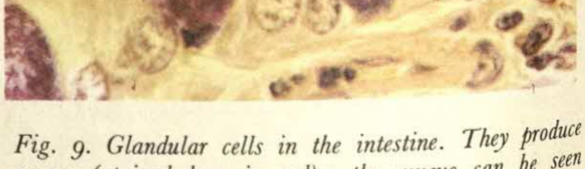

Fig. 9. Glandular cells in the intestine. They produce mucus (stained here in red); the mucus can be seen coming out of one of the cells (mag. × 920).

structure with the electron microscope, and their composition and enzymatic behaviour after they have been isolated by centrifuging.

The chrondriosomes

Mitochondria are small structures, from 0·2μ to 8μ long, shaped like granules or filaments. They are fairly uniformly distributed throughout the cytoplasm, except in those cells which carry out special functional activities, where they are found in the zone of synthesis. The mitochondria are motile, showing characteristic movements and changes of shape which are very probably linked with their functional acrivity (Fig. 5 and 6). It can also be demonstrated that the shape and size of the mitochondria are related to cellular activity. A remarkable fact is that whatever the size and number of the mitochondria in a particular cell, their total amount remains fairly constant, for when they are experimentally damaged or put out of action, the cell reforms new ones so as to re-establish the original quantity of functionally intact material.

The structure of the mitochondria appears fairly complex when examined under the electron microscope. Each rod is surrounded by an extremely thin double membrane, from which a series of ridges arise at intervals and point towards the inside (see Fig. 4, page 43).

Numerous investigators have succeeded in separating mitochondria from the cell by fractional centrifuging,[8] in order to study their composition and biochemical properties. One of their striking features is their remarkable enzymatic richness, and their activity accounts for as much as 50 per cent of the total cellular activity. Their chief enzyme systems are those which carry out respiration or take part in providing energy. Many other vitamins and co-enzymes are also found.

These bodies may therefore be regarded as the respiratory and energy centre of the cell. But as well as carrying out these fundamental processes common to all cells, they are closely concerned with special activities peculiar to certain types of cells, such as the synthesis of particular structures or secretory products. When living cells are studied by accurate methods, such as the analysis of 'stills' from a microciné film it is found that the mitochondria do not develop as independent structures in the cytoplasm. On the contrary, they maintain important functional relationships with other cell components, such as the nucleus, nucleolus and basic cytoplasmic matrix, which are accompanied by exchanges of substances.

Ergastoplasm and the Golgi material

Under an ordinary microscope the basic cytoplasmic material in which the mitochondria develop, appears to be optically homogeneous. However, under favourable conditions and with more elaborate optical systems, some structures can be made out at the limits of visibility. The high resolving power of the electron microscope enables us to tackle this problem, but unfortunately, only fixed, that is dead, cells can be studied by this technique and it is sometimes difficult to tell how much, if at all, the structures have been altered by fixation. Nevertheless, the electron microscope shows up submicroscopic lamellae in the cytoplasm of some cells, particularly those of the glands. These are composed of a system of double membranes and very minute granules. The system comprising all the lamellae in the cell is often called the endoplasmic reticulum (Fig. 7). In other cells, such as the macrophages, there is usually a system of fine granules, the microsomes, which may be either scattered or arranged in a network.

In living cytoplasm a special somewhat rounded zone often enclosing fat droplets and several vacuoles may be shown up clearly by staining with neutral red. The mitochondria are frequently grouped around this region, which is usually situated near the nucleus (Fig. 3). This is the Golgi zone. In preparations which have been fixed and coloured by special techniques, it usually appears as a network which varies in size and shape according to the type of cell. Its significance is uncertain, although it may be the site of certain substances directly concerned with cell synthesis, particularly during glandular secretion.

The cell nucleus

So far we have only considered the cytoplasm and we must now turn to the other essential cell component, the nucleus. Its shape is usually related to that of the cell itself: spherical in round cells, and elongated along the axis of long cells. Its size is usually proportional to the size of the cell, while there is a definite relationship between the volume of the nucleus and that of the cytoplasm: this is the nuclear-cytoplasmic ratio which ranges on average from 1/7 to 1/10.

In living cells examined by the phase contrast microscope, the nucleus appears as a fairly uniform region delimited by a fine membrane, the nuclear membrane, and en-

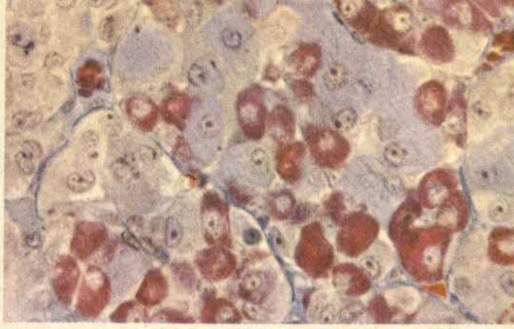

Fig. 10. Section of the front lobe of the pituitary gland showing characteristic features of an endocrine gland. The anterior lobe of the pituitary is made up of several cellular types each of which produces different hormones. These cellular types can be distinguished from each other by their affinity for certain staining substances (mag. × 440).

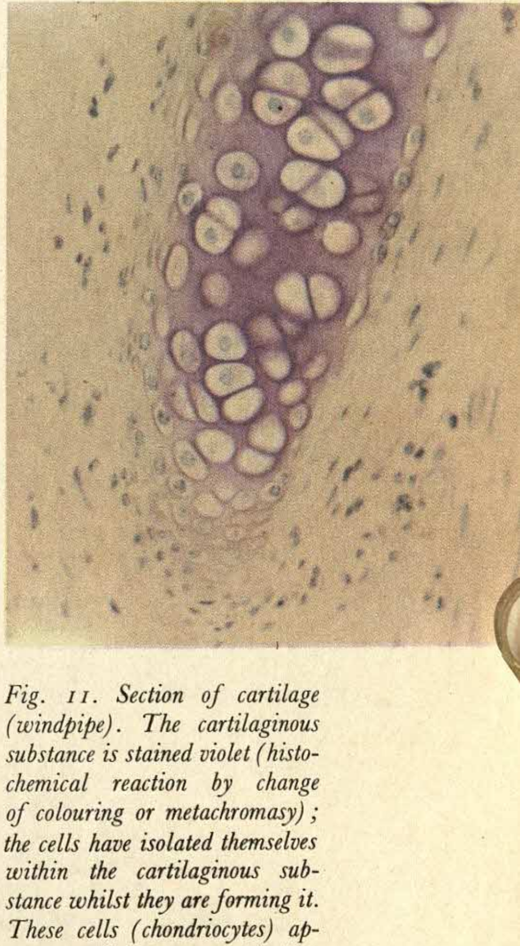

Fig. 11. Section of cartilage (windpipe). The cartilaginous substance is stained violet (histochemical reaction by change of colouring or metachromasy); the cells have isolated themselves within the cartilaginous substance whilst they are forming it. These cells (chondriocytes) appear as light spots with blue nucleus. All round the cartilage is connective tissue (mag. × 250).

Fig. 12. Mitosis of a chicken's fibroblast cultivated in vitro *(fixed and stained). 36 characteristic chromosomes of the chicken can be distinguished (black bands) (mag. × 2200).*

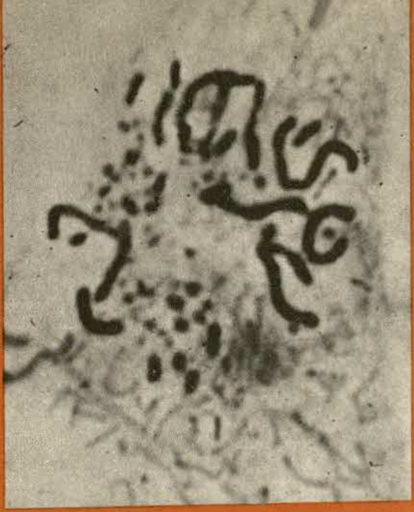

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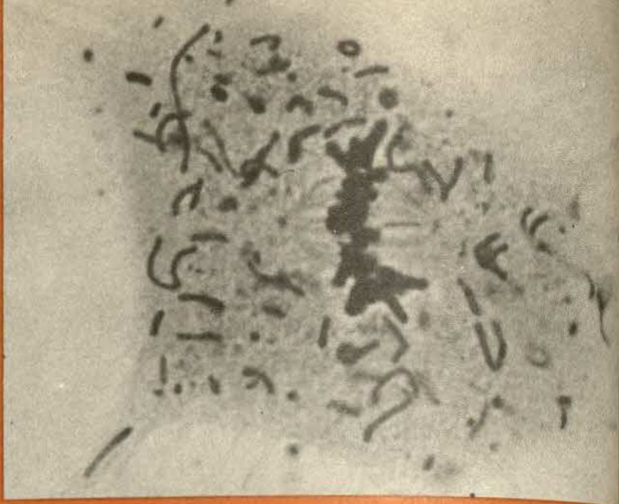
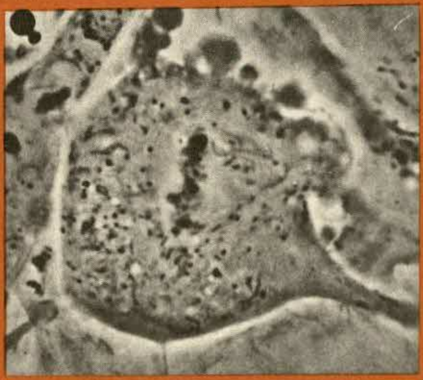
Fig. 13. Metaphase (cell fixed and stained). The chromosomes are grouped in an equatorial plate along the axis (mag. × 2600).

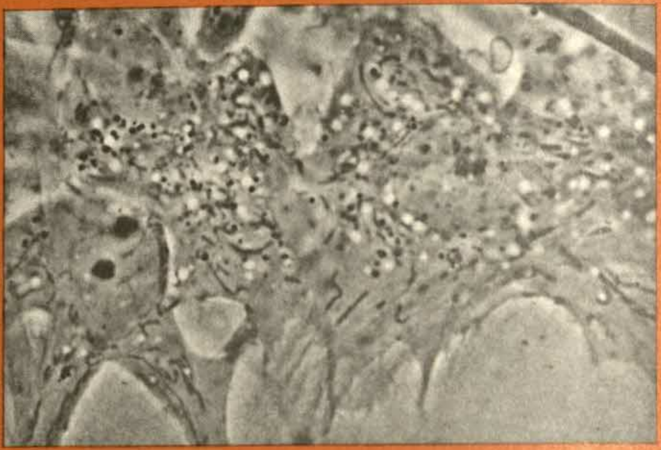


13

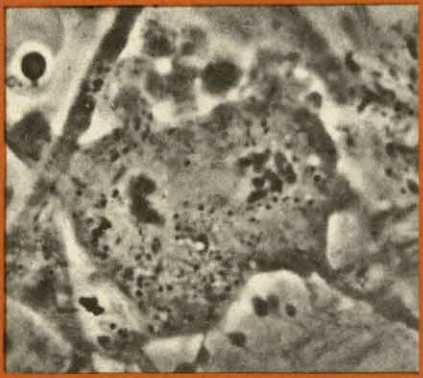
Fig. 14. Three successive stages of mitosis in a fibroblast cultivated in vitro. *Photographs in phase contrast: (a) metaphase; (b) anaphase; (c) division has taken place and the two daughter cells are now separate (mag. × 1200).*

14 a



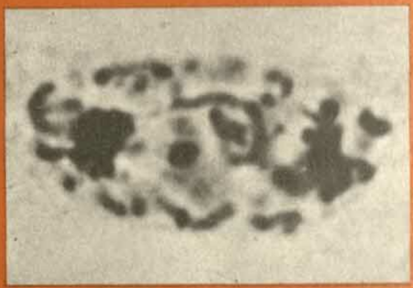


14 c

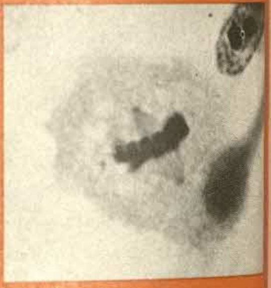


14 b

Fig. 15. Various stages of mitosis in a culture treated by alkaline phosphatase. (Chicken fibroblasts).
(a) Prophase. The nuclear membrane has disappeared. The reaction is positive at the level of the nucleolus (black spot) and the chromosomes (mag. × 3200).
(b) Metaphase. The chromosomes are grouped in equatorial plates, seen here in profile; the axis shows up clearly (mag. × 1500).
(c) Another mitosis. Note the characteristic appearance of beads and strips, visible in some chromosomes (mag. × 2000).
(d) Anaphase (mag. × 2000).



15 a



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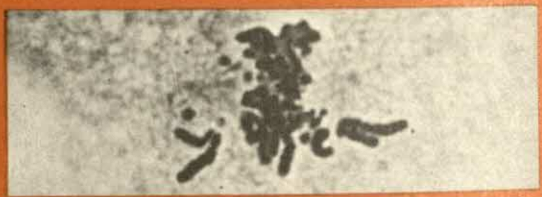

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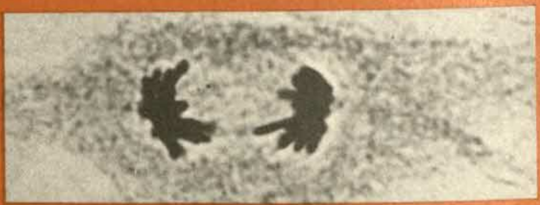
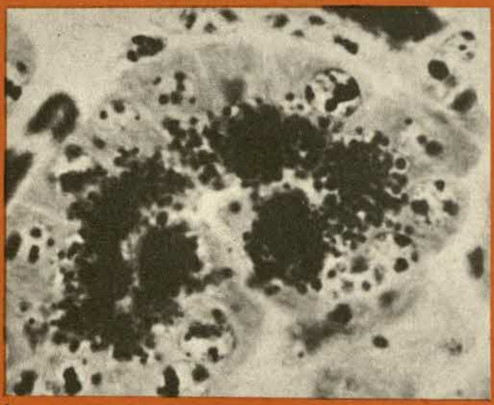


15 d

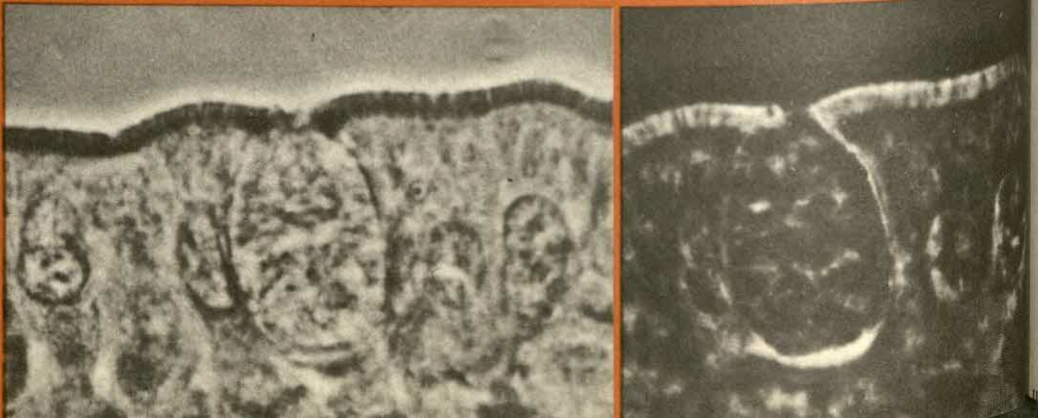
Fig. 16. Simple prismatic epithelium with pitted surface. Unstained section of small intestine; the same place was photographed in phase contrast normally (to the left) and in negative phase contrast (right); the cells of the intestinal epithelium are absorbent; the pitted structure shows up well in the right hand picture; the large cell is one which produces mucus (mag. × 1750). (See also fig. 9).



16a

Fig. 16a. Glandular epithelial cells of the pancreas. Appearance typical of an external secretory gland. The cells contain secretory granules (stained black) which they have formed and will ultimately excrete (mag. × 1280).

16



closing a number of granules which are often very small, of varying distinctness and uniformly distributed. These are the chromatin granules which can be seen better after fixing and staining, on account of their affinity for basic dyes. They are composed primarily of nucleic acids (DNA) and include a considerable amount of histones and probably enzymes as well, in particular, alkaline phosphatase.

Also present in the nucleus are one or two heterogeneous masses, the nucleoli, which show opaque patches alternating with more transparent regions. Like the chromatin granules, the nucleoli have a high affinity for basic stains and contain a high quantity of the other nucleic acid, ribonucleic acid (RNA).

One of the essential functions of the nucleus is to transmit the hereditary characters. But, apart from this the nucleus actively participates in the general metabolism of the cell and probably also in the synthesis of proteins. Experiments with nuclear grafting show that the nucleus is necessary both for its direct and indirect effects.

Mitosis: an essential cell function

If we continue to watch the fibroblast described above, we find that after a few hours the nucleus changes: the cell is beginning to divide, that is, to undergo mitosis. This is another essential characteristic of life, reproduction.[9] Briefly, the process takes place as follows: the structure of the nucleus changes, threads, which are the chromosomes,[10] become visible and thicken. The latter are seen particularly well in fixed and stained preparations; in living cells under the phase contrast microscope (Fig. 14); or after certain histochemical reactions, such as that for alkaline phosphatase (Fig. 15 a to d), or the Feulgen reaction for deoxyribonucleic acid (DNA) (Fig. 8).

The chromosomes carry all the DNA in a cell and every cell of an organism belonging to a particular species contains exactly the same number of chromosomes, except for the sex cells. That is, the sperms and eggs, which are only 'half-cells' and have half the typical number. The number of chromosomes is one of the characteristics of a species, thus there are 46 in man, 36 in a hen, and 20 in corn; the number is always even, since there are two groups, one coming from the father and the other from the mother. These two parental sets carry with them the hereditary factors or genes which are passed on from one generation to the next.

At the same time as the chromosomes appear in the cell, the membrane surrounding the nucleus disappears in addition to the nucleolus. This stage is called prophase (Fig. 15a). Each chromosome then splits lengthwise so that the DNA of the original cell, that is the hereditary material, can be equally distributed between the daughter cells. It might be thought that at each division the amount of these important molecules would be reduced by half and after a certain number of mitotic divisions there would be hardly any left. This does not happen, because before dividing the fibroblast synthesizes DNA, so that just before mitosis each chromosome carries a double amount of DNA which is distributed equally to the two daughter cells. The synthesis of DNA is one of the fundamental processes of growth and cell division and may be studied either by cytophotometry or histoautoradiography[7] (Fig. 1).

If we continue to watch the dividing cell, the chromosomes, which have split progressively, but not yet divided into two, arrange themselves along the equator of the cell. Seen from above, this looks like a sort of star with a clear centre and with rays formed from the chromosomes. Seen from the side it looks like a thick irregular band (Figs. 13 to 15). Thus the equatorial plate results from the grouping of the chromosomes in the middle of the cell.

This stage is called the metaphase. At the same time, a clear spindle-shaped space has formed. By using special staining techniques or examining the cells under polarised light, very fine threads can be made out to which the chromosomes are attached. These fibres play an important role in division, since they draw each half of the already doubled chromosomes to opposite ends of the cell. This process of distribution of the chromosomes and their movement and displacement towards the ends of the cells is the anaphase (Figs. 14 and 15). At the same time the cell shows a central constriction and begins to separate into two. Separation is completed. This is the telophase.

Each of the daughter cells is identical to the cell it came from, and has, in particular, an identical chromosome set. The chromosomes disappear progressively, while the nucleus, together with its surrounding membrane and nucleolus, reforms.

The whole phenomenon of cell division brings many complex processes into play. Clearly these must be effective, not only for the successful completion of mitosis, but in

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(4) *On the culture of tissues and more particularly the experiments carried out by Carrel and Ebeling, see the chapter: 'Culture of tissues in synthetic environments'.*

(5) Phase-contrast microscopy: *Discovered by Zernicke (1935) who was awarded a Nobel prize, microscopy in phase contrast makes it possible to distinguish fine structures of colourless or coloured but only slightly contrasting objects. The eye can only distinguish contrast of amplitude or colour (ordinary microscope); microscopy in phase contrast is an optical device by means of which alterations in the phase of light, due to differences in the refractive index of the object, are changed into directly perceptible amplitude contrasts. This is particularly valuable for the study of living cells, where the different structures are clearly shown without being damaged and with great clarity. Microscopy by interference, still more recent but based on a similar principle, enables small variations of light phase to be measured; it is thus possible to determine the concentration of certain substances in the structures observed.*

(6) Microcinematography: *Using microscopy in ordinary light, on a black background, in phase contrast, or interference, microcinematography is the favourite method in studying the behaviour of living cells or some of their components 'in time'. By taking shots at ordinary, accelerated, or reduced speeds, this technique has made it possible to tackle many problems of cellular biology: it is particularly valuable when it is completed by the analysis of the films shot by shot, according to very special and precise methods.*

(7) Histoautoradiography: *This technique is based on two facts. The first is that particles or rays emitted by a radioactive element can impress a photographic emulsion; the second is that the living cells seem to make no distinction between an ordinary substance and the corresponding substance containing a radioactive element (a 'marked' or 'labelled' radioisotope) which they incorporate in the same manner. Thus the utilisation of a substance by a cell or tissue can be followed, as well as its distribution and turnover. The technique consists in covering the histological section or culture of the tissue by a photographic emulsion in close contact with it and this is developed after a few hours or several weeks exposure in a dark room, then set up as an ordinary histological preparation (Fig. 1). The position of the silver granules shown up in the print corresponds to the localisation of the marked substance. The radio-elements which lend themselves best to the labelling of organic molecules are heavy hydrogen (tritium), calcium, phosphorus, iodine, sulphur, nitrogen, iron, etc.*

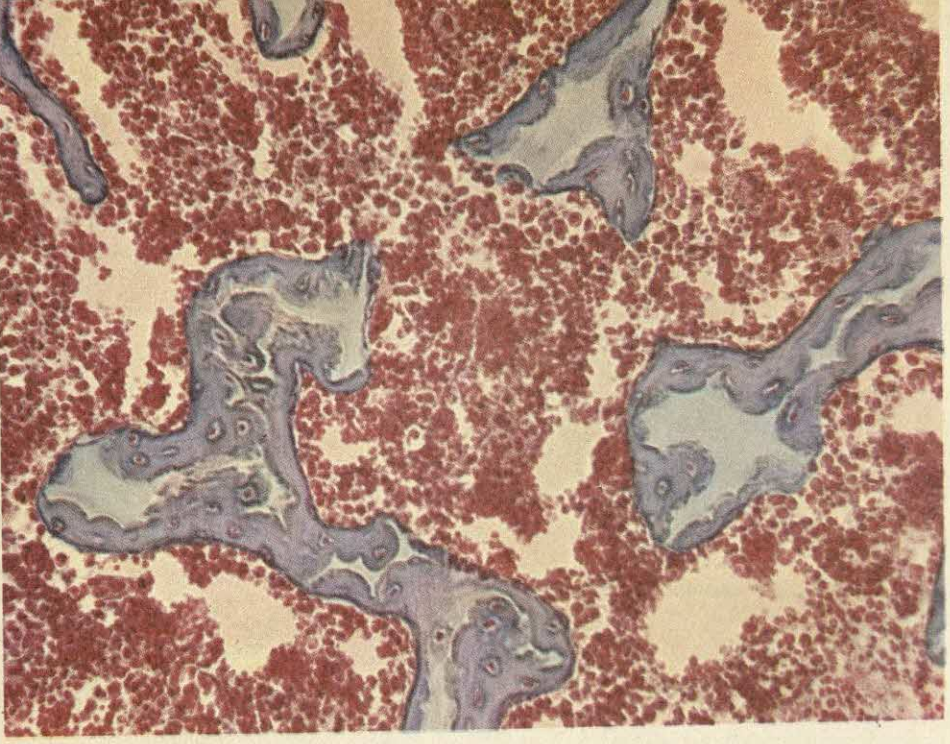

Fig. 17. Spongy bone (compare with fig. 22 showing a compact bone). Section of the rib of a human foetus at birth. Bone shown in blue; bony substance pale blue; the cells (osteoblasts) producing this substance are stained dark blue. Between the bony material, which forms a kind of sponge, lies the marrow. This, stained red, is a blood-forming organ, producing red corpuscles and some white ones (mag. × 270).

(8) Differential centrifuging: *All cellular components do not have the same density: they react differently when submitted to a high centrifugal force which separates one from the other. Previously crushed and dissociated tissues or broken cells are placed in a suitable liquid, then revolved at a great speed (20,000 rpm or more) at a low temperature. This technique separates the various components of cells which can then be studied and analysed 'in fractions'.*

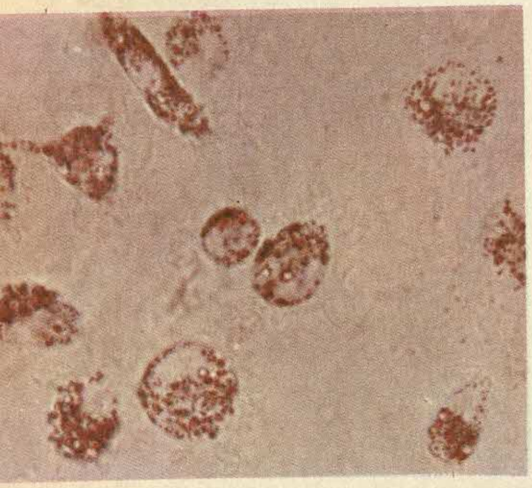

Fig. 18. Vital stain (neutral red) in living macrophages. This is a selective vital stain; only the macrophages become red. Other cells are hardly touched and are invisible here (mag. × 700).

order for the cell to prepare to divide. Among the most important processes are the following: the provision of energy, which is primarily the role of the mitochondria; the synthesis of DNA before division; and the correct functioning of the spindle. Interference with any of these mechanisms will upset or prevent division. In short, the cell is particularly vulnerable during division and is then most sensitive to the action of both chemical and physical agents.

Temperature is known to affect mitosis. It takes place most rapidly in the higher vertebrates at around 42°C, but soon several anomalies appear. Below 24°C and above 45°C, mitoses that have already begun are not completed and no other cells start to divide. X-rays, and the rays from radium have a special effect in that a sufficient dose kills dividing cells. This property is used in human medicine, particularly in the treatment of certain tumours.

Mitotic poisons

One group of substances is particularly interesting because they are capable of upsetting or inhibiting mitosis in weak concentrations, although apparently they do not affect the nuclei of neighbouring cells of the same type which are in the resting phase. While some of these substances are unspecific in their action, others are selective, or nearly so, and act on mitosis at a particular stage, either by interfering with one of the steps or by affecting some particular part of the cell. These substances are clearly of considerable pathological interest and are also important from the point of view of cell biology, because they help us to find out about the mechanism of normal mitosis.

According to which process is affected by mitotic poisons, the following may occur:

(a) *Cells about to divide are inhibited.* (Preprophasic inhibition). Cells are prevented from entering into mitosis particularly by those substances which act on the mitochondria, such as trihydroxy-N methylindole and adenosine triphosphate, thus showing the importance of mitochondria in preparing the cell for division.

(b) *Cell division stopped at metaphase owing to inhibition of the spindle.* Some substances, such as colchicine and antagonists of folic acid, prevent the equatorial plate from separating into two, thus blocking mitosis at this stage.

(c) *Defective chromosome separation.* Chromosomes[10] appear to 'stick' in anaphase and reach the ends of the cell with difficulty, as after treatment with trypaflavine.

(d) *Fragmentation of chromosomes.* This is brought about by the action of mustard gas, or other analogous substances, such as mustine, used in the treatment of certain leukaemias.

(e) *Two daughter cells prevented from separating.* A single giant cell with either two nuclei or a single giant nucleus is formed. It is probably brought about by substances which modify the viscosity of the cytoplasm and possibly some of the respiratory processes.

Specialised cells form tissues

An adult vertebrate is composed of an amazing variety of different cells. However, they all have some characters in common and these are the ones just outlined in describing a very simple cell, the fibroblast. But while all the cells resemble each other in that they are constructed, live and multiply in much the same way, they are distinguished from one another, because they carry out some special function besides the basic activities essential to their own existence. It must not be forgotten that all the cells in an organism are derived from a single cell, the fertilised egg. During embryonic development, the cells differentiate, that is, there is a division of labour between the cells and they become specialised. This affects their structure, composition, and function.

A group of cells which have fundamentally the same function and structure constitute a tissue. In order to classify tissues, we must therefore take into account, not only morphological characters as seen under the microscope, but also functions. On this basis four large groups of tissues may be distinguished: covering and glandular epithelial tissue; various types of connective tissue; smooth and striated muscle tissue;[11] and nervous tissue.[12]

As an example we will describe briefly the epithelial and connective tissues. These are the most widespread and polymorphic tissues, and also the first to be formed both embryologically and phylogenetically.

The epithelial tissues

Epithelial tissues are composed almost entirely of cells. Usually, these have a simple, regular, well-defined shape. They are in contact with one another over large surfaces, in contrast with the connective tissue cells, which are separated by varying amounts of interstitial material. The epithelial cells form compact groups or continuous membranes. Blood vessels or capillaries are never found in epithelia, but nerve fibres and migratory cells may be present. They are classified into two main groups: the covering epithelia, which are simple protective membranes, such as the skin, and the glandular epithelia, which synthesize the secretory substances used by the organism. However, there is no hard and fast distinction between the two types.

Covering epithelia essentially form a continuous layer which covers the external surfaces of the body or lines its internal cavities. There are two main types according to the number of their cell layers: simple or many layered.

Epithelia are named according to the shape of the cells nearest the surface. Thus there are pavement epithelia, with flat cells like paving-stones, cubical epithelia, and prismatic epithelia (Fig. 9). However, these distinctions are only of value for classification, for the epithelial tissue is very flexible and its cells readily change shape under mechanical stress.

Some covering epithelial cells have special structures on the surface which is in contact with the surrounding environment. In particular, there are many with short vibratile cilia, which move fluid or mucus along the surface of the epithelium. These are found in the lining of the nasal and bronchial tracts and the Fallopian tubes. Single motile flagella may occur and also stereocilia, which are thin short immobile threads. Some of the cells which are adapted to sensory functions have one or more stereocilia, and these are concerned with sensory perception, such as the cells of taste, smell and hearing. Yet, another type of differentiation of the peripheral edge of the cell is the striated or brush border characteristic of the absorptive cells of the small intestine (Fig. 16).

The cells which make up the covering epithelia are sometimes held together by special structures. Thus in stratified pavement epithelia, which must resist strong mechanical stresses, the cells are joined together by a system of fibrils which pass from one cell to another. In some prismatic epithelia, the cells are firmly held together at their outer edge by the framework that unites them. These may be compared to a sort of trellis, into whose mesh the cells are inserted.

Epithelia always rest on connective tissue, either directly, or more often they are joined to it by a thin transparent membrane. This basal membrane is firmly attached both to the epithelial cells and the underlying connective tissue. It is the latter which nourishes the epithelium, by providing it with nutrients and oxygen.

Glandular epithelia: in theory every cell is capable of synthesizing a variety of products. It is able to draw nutrients from the surrounding environment, use them and reject the waste. But some cells specialise: these are the gland cells. They synthesize special substances which accumulate in the cytoplasm in the form of secretory granules (Fig. 16a). They then excrete the synthesized products (merocrine secretion). In some cases the whole cell constitutes the secretory product and the substances formed do not leave the cell, which is then rejected with the contents it has synthesized (holocrine secretion).

Usually gland cells have a large nucleus, a well-developed Golgi apparatus and abundant mitochondria. As we said earlier, the mitochondria and Golgi apparatus play an important part in secretion. The activity of the cells is accompanied by cytological changes which vary in clarity and characteristics according to the phase of secretion.

The arrangement of glandular tissue is very diverse. The cells may be arranged in membranes, or form blind sacs of varying complexity, or be massed into epithelia.

Many glands pass their secretory products to the exterior, either directly or via excretory tubes of various types. These are the glands of external secretion, such as the salivary or digestive glands. In contrast, there are other

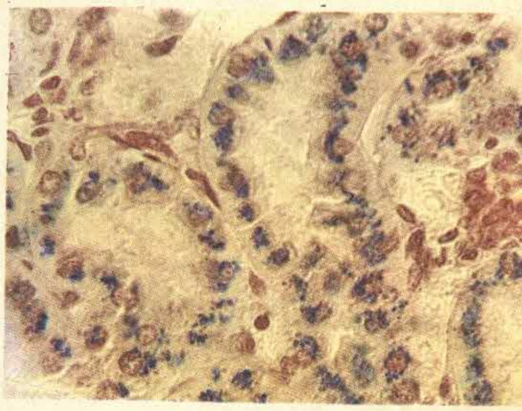

Fig. 19. Vital stain in the kidney cells (fixed preparation). Renal tubes seen here in section. The cells (cytoplasm stained pink, nuclei red) which form the wall of these tubes have picked up some of the stain (blue granules) which has been injected into the animal, passed through the glomeruli (filtration), and been reabsorbed at the level of the tubes (mag. × 680).

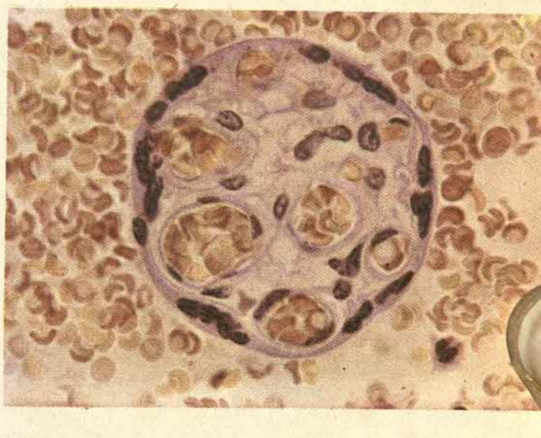

Fig. 20. Section of human placenta. The villus (round pink mass) plunges into the maternal blood, of which red corpuscles can be seen (stained orange). Inside the villus, small vessels can be seen in cross-section containing other red corpuscles: these are those of the foetus. The exchange of material (oxygen and glucose in particular) is made through the walls of these small vessels and the very thin wall of the villus (mag. × 425).

glands which have no excretory ducts but pass their secretions directly into the blood or lymph. These are the glands of internal secretion, or endocrine glands, such as the pituitary, thyroid and adrenals (Fig. 10).

The connective tissues

A vertebrate embryo is made up of organ rudiments, together with a cell system composed of star-shaped cells, the fibroblasts. These are separated from each other by a fluid substance in which some fibres manufactured by the cells are found. This is the mesenchyme, the embryonic connective tissue from which all the other types of connective tissue are ultimately derived. There are many types, but their fundamental characteristic is that they are formed from the same three components: free cells, an amorphous interstitial material (the basic matrix), and fibres. The free connective tissue; the skin (Figs. 12 a and b); cartilage (Fig. 11); bone (Figs. 17 and 22); tendons; muscles and so forth (Figs. 23 a and b) are the connective tissues which differentiate. They vary according to the nature of the cells and the type, amount and arrangement of the fibres and according to the composition and consistency of the basic matrix. It should be noted that blood and lymph are also connective tissues and may be regarded as possessing a fluid basic matrix.

One of the characteristic properties of connective tissues is that they may be substituted for one another. This takes place both during the evolution of the species, as when membranous supporting parts become cartilaginous and then bony, and during the development of the individual; for even in an adult bony nodules develop with age in the cartilages of the larynx. Some types of connective tissue change as the fibroblasts that surround cartilage changes progressively into cartilaginous cells.

Connective tissues have many functions. Some of them support the blood and lymph vessels which carry oxygen and nourishment to the epithelial tissues. Others are adapted to special mechanical functions, such as bone, tendons and cartilage. Mechanical factors play an important role in their development, since stresses and strains influence the amount, arrangement and orientation of the fibres. Others again provide the mother cells of the formed elements of blood, or are blood-corpuscles or important agents in the defence of the organism.

Indeed, there are some connective tissues which provide the main defence mechanisms of the organism. Thus one of the types of white blood corpuscle, the polymorphonuclear leucocytes, may leave blood vessels, absorb microbes and ingest them (Fig. 24). They are motile (Fig. 27), sensitive to factors summoning them to the sites of infection, and rich in enzymes capable of digesting germs. Another type of cell which plays an important part in the organism's defenses is the macrophage (Figs. 18 to 25). These have very diverse origins and come from the circulating blood or from the transformation of fibroblasts within the tissues, or even from other cells. Like the leucocytes, the macrophages are capable of destroying germs by phagocytosis, but can tackle larger prey as well, such as dead or degenerate cells, old red blood corpuscles, and particles of carbon or dust.

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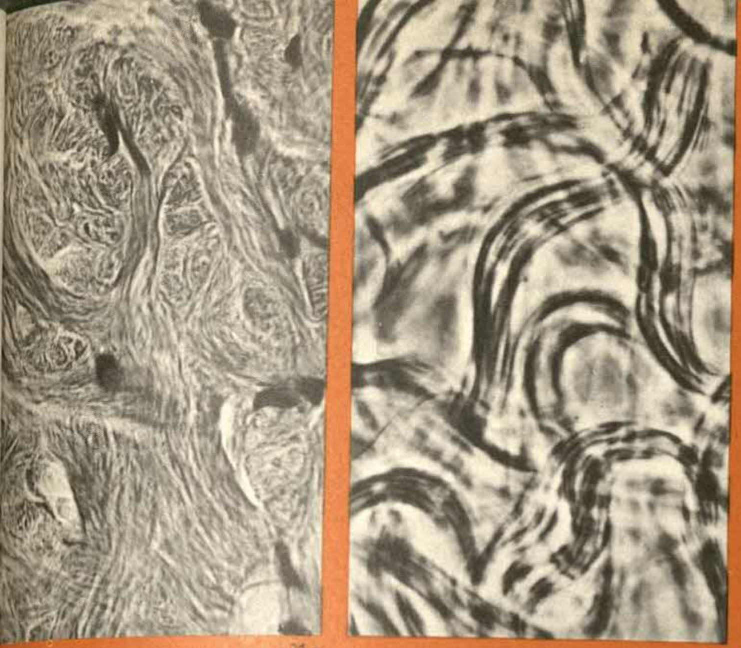
(9) Mitosis and growth: *If one remembers that the body of an adult animal contains several thousand million cells, all derived by successive divisions from a single fertilised egg-cell, one is led to believe that, to reach this result, a great many successive mitoses must be required. In fact, this is not so. From a strictly mathematical point of view, it is a simple geometric progression. Let us take an organism made up of* 10^{14} *cells (100,000 thousand millions) and let us call the number of divisions necessary X. As the number of cells doubles with each partition, the number of divisions is obtained from the equation* $2 = 10^{14}$; *this gives* $x = 47$ *(Gamow, 1955). In other words, each cell of the organism is a member of the approximately fiftieth generation of the primitive egg. Of course, no account is taken here of the tissues which are being constantly renewed.*

(10) *On chromosomes, see the chapter 'Problems of Heredity' by Pierre Joly.*

(11) *Although all protoplasm can contract, this property is greater in the muscular cells; muscular tissue is specially adapted to the function of contracting. The contraction takes place in differentiated elements of the cell and always in the same direction, along the length. These greatly differentiated elements in the cell are special fibrils—the myofibrillae—visible under the microscope. They are of two kinds: smooth (homogeneous) or striped (heterogeneous); the first appear to be without structure under the microscope, whilst the others have stripes of lighter (isotropic) and darker (anisotropic) 'discs' of a fine and complicated structure all along their length. According to the type of fibrils, there are smooth muscles which contract slowly and independently of the will (involuntary muscles) and striped muscles which contract more quickly voluntarily (Figs. 23 a and b).*

(12) *The nervous tissues are made up of different elements which are, on the whole, specialised in the perception and transmission of various stimuli. Nerve cells—or neurones—are often rather large with extensions of considerable length. By means of these extensions they are connected with other neurones, or other cells, sometimes situated a long way from the original neurone; without these, no real nerve function would be possible. Another characteristic of neurones is the presence in their cytoplasm and extensions of fine fibrils which can be shown up by special methods (Fig. 26).*



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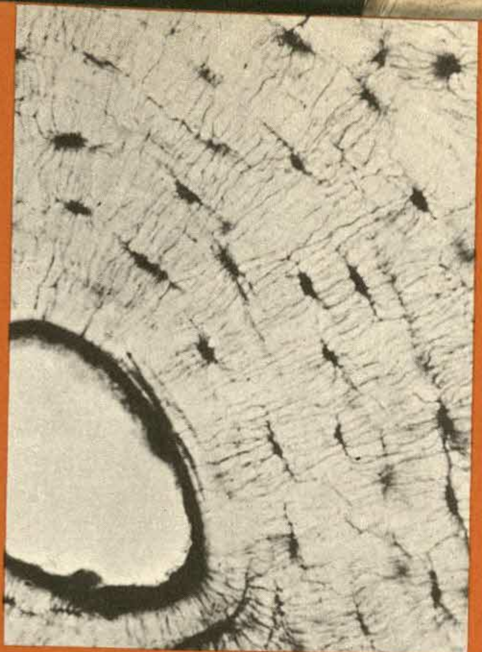

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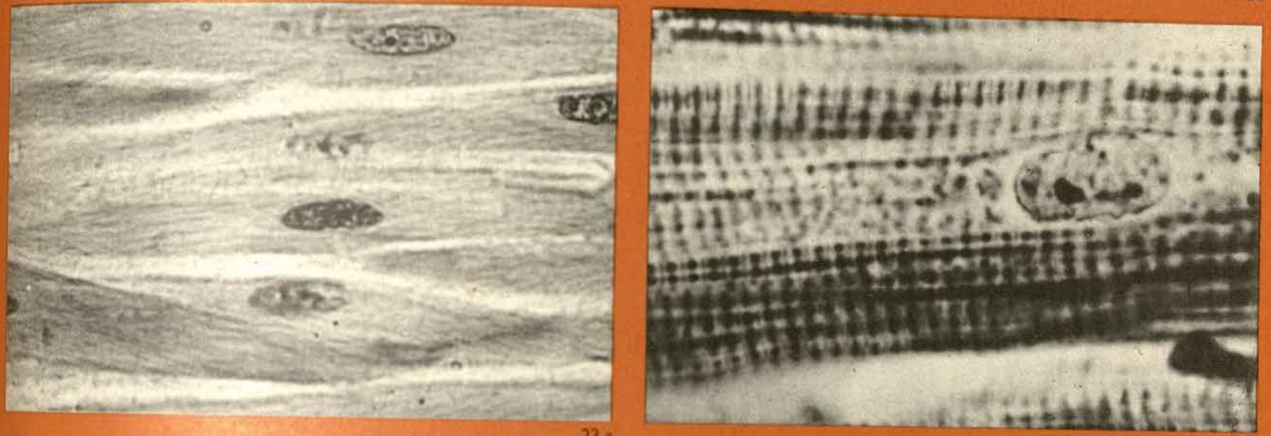
Fig. 21. (a) Connective tissue of the skin. The cells, of which the nuclei are mainly seen, are separated by the ground substance and by abundant fibres grouped in batches (mag. × 580). (b) Living dermis, photograph taken in phase contrast. Collagen fibres intertwined and formed by closely-packed fibrils (mag. × 1400).

Fig. 22. Section of bone. The cells (black stain) buried in a ground substance rendered rigid by impregnation with salts of calcium intercommunicate between themselves and the blood capillaries by tiny canaliculi. They create the ground substance and numerous fibres invisible here (mag. × 300).



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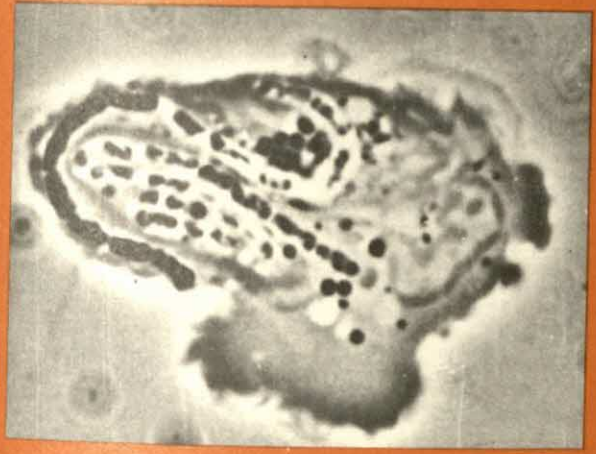
23 b

Fig. 23. (a) Section of smooth muscle, cut longitudinally (muscular coat of the intestine). The muscular cells are fusiform and contain smooth myofibrillae difficult to see here (mag. × 800). (b) Fibres of striped muscle. The myofibrillae are heterogeneous and are piled longitudinally against each other, side by side with isotropic and anisotropic discs. This gives the effect of transverse stripes (mag. × 600).



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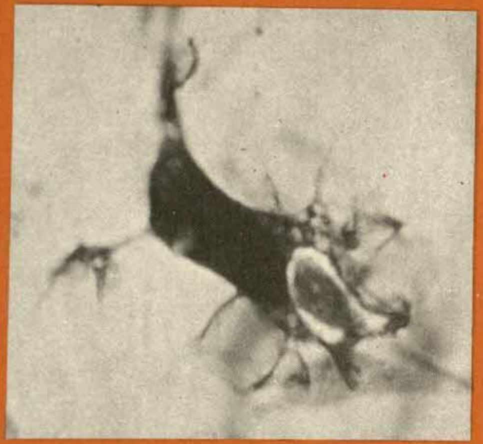

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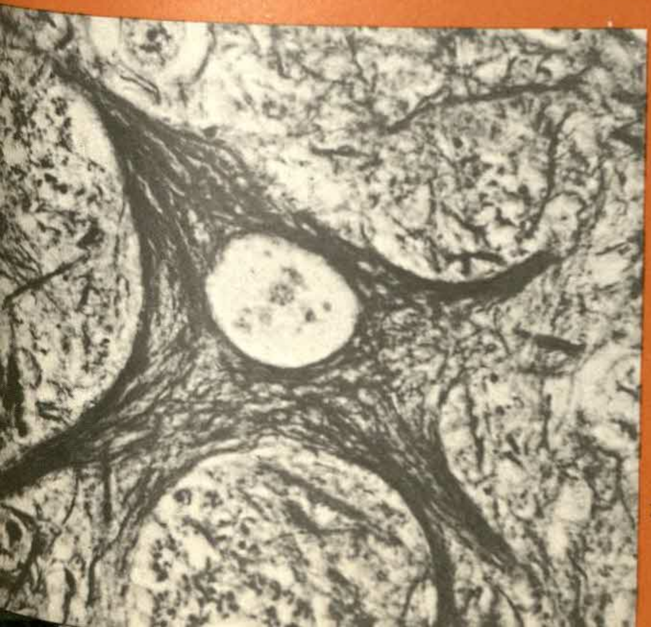
Fig. 24. Living white blood corpuscle, seen in phase contrast. The thick more or less continuous black marks are bacteria absorbed by phagocytosis and being digested (mag. × 3300).

Fig. 25. Macrophage (tissue in culture, fixed and stained). Another kind of cell which helps to defend the organism; the macrophage is in the tissues where it plays the role of 'cleaner': it is characterised mainly by the presence of 'wavy membranes' (mobile peripherical veils); the macrophage shown here has phagocytosed an old red blood corpuscle (dark grey spot in the light vacuole) (mag. × 1100).



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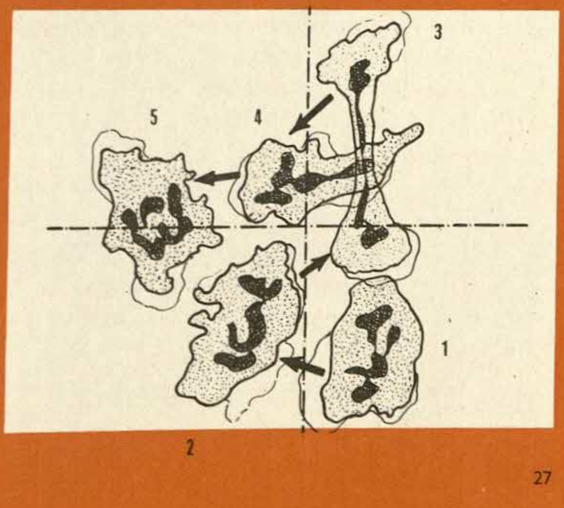

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Fig. 26. Cell body of a neurone; the nucleus can be seen (light spot) and the beginning of the extensions, which give a star-like appearance to the cell; the neurofibrils are visible as thin black filaments in the cell (mag. × 1000).

Fig. 27. Movement of a polymorphonuclear white corpuscle. Drawings of a single cell from a film; the cell changes shape actively as it moves on a solid substratum (direction indicated by arrows and numbers); interval between drawings respectively 60, 45, 75 and 60 seconds.

The cultivation of animal and plant cells in synthetic nutrients

Philip R. WHITE

HISTORICAL NOTES

1865
W. KNOP defined the salt requirements for agricultural plants.

1902
G. HABERLANDT formulated the idea of cultivating isolated plant cells as a means of studying the phenomena of life.

1907
R. G. HARRISON solved an important biological problem concerning the origin of nerve fibres, by growing frog nerve cells for short periods in a complex culture serum.

1910
M. R. and W. H. LEWIS grew a number of isolated animal tissues for short periods in defined solutions.

1911
A. CARREL demonstrated the potential immortality of heart cells when grown outside the body in complex media.

1934
P. R. WHITE demonstrated the potential immortality of tomato roots in a nutrient defined except for 0·01 per cent of yeast extract.

1939
P. R. WHITE, R. J. GAUTHERET and P. NOBÉCOURT established cultures of a variety of plant tissues in completely defined nutrients.

Continued on next page

Organisms—plants and animals—are complex societies of cells. Each cell, as a member of its particular society, is a distinct individual. Theodore Schwann pointed out more than a century ago (1839) that it has the same sort of individuality as does a bee in its swarm. A solitary bee cannot reproduce itself nor long survive: only the swarm is capable of a complete existence; each bee is an individual, but in a limited sense only. Cells have this sort of limited individuality.

A cell is: (1) what it has been from the beginning (heredity), (2) what it has taken in (nutrition), and (3) what it has experienced (developmental history).

Since all the cells which make up the society which we call 'an organism' have arisen from a single fertilized egg (Figs. 2A and A^1) by a series of divisions, it is clear that all the individual members of the complex began with identical capacities. The ultimate differences which we see between a ciliated cell of the lining of the lung (Fig. 2 D^1) and a bone cell (Fig. 2 C^1) or between the hair cells of a root (Fig. 2 B) and the stony cells of a fruit (Fig. 2 C) have arisen somewhere along the road by which the egg has become a man or a tree. Many of these differences, though perhaps not all, are merely the result of the positions in which cells find themselves, their interaction with their neighbours. One of these interactions is the process by which parts of a system determine the sort of nutriment which reaches other parts, other cells, from the raw supply provided to the whole. A bee becomes a queen or a lowly worker according to the food doled out to the uncompleted larva by a previous generation of workers. If we are to understand a cell we must know a great deal about its nutrition.

Descartes taught us that problems could best be solved by breaking them down into their simplest elements. One way of studying organisms is to take them apart, into their constituent cells, to reduce the environment of each cell to its simplest effective elements, to determine what in the normal environment is essential, what lethal, what beneficial or detrimental without being either essential or lethal, and what merely fortuitous. In our final Utopia we must retain the first, eliminate the second and last, and introduce the third with discretion. In what we will discuss here we will limit ourselves to only one aspect of the whole, namely that represented by the statement: 'A cell is what it eats.'

Gastronomy and dietetics

There are two ways to find out about foods. The gourmet may go to a restaurant, order the finest meal available and over his liqueur discuss, on the basis of sight and smell, touch and taste, that is, by the methods prosaically called chemistry and physics, what were the constituents of his menu. Even when he has arrived at the most precise and detailed answer possible by this means he can still tell us nothing about which parts of the dinner were essential for which functions, and which were merely the pleasant sauces and unnecessary roughage. Another method is to bring in from the fields and shops, and still better from the chemist's shelves, one at a time and in various combinations, a great many of the simplest possible foodstuffs and, by trial and error, determine which are best utilised, in what amounts, and what is the smallest number and quantity of such stuffs on which one can live comfortably and function efficiently. This method is tedious and faces many a set-back, but once an unequivocal answer is obtained it is an immediately useful and complete one. Both methods have been used in the study of the nutrition of cells.

Fifty years ago (1905) anatomists were faced with a puzzle: 'Where do nerve fibres come from? Do they grow out from the brain, or do they grow from the innervated member such as a muscle, or from both, or are they laid down in some way in the intervening ground tissues?' After all, one cannot tell from looking at a telephone wire whether it was laid down from the phone end or from the exchange, and few indeed, on seeing a wire, would be so foolish as to suggest that it had been made by the exchange and not in a factory in another city. It fell to the lot of an American, Ross G. Harrison (1907), to find a clear answer to this question. He did so by following Descartes's advice, by taking an animal apart, by growing a bit of nerve in a drop of clotted lymph. Here he could watch the fibres being spun out from the ganglion cell, the wire being laid down from the exchange (Fig. 2 B^1).

But watching the spinning of a nerve fibre out of the stuff taken from a clot of lymph tells us nothing very precise about the nutrition of a nerve cell. These cells survived at best for only a few days. Harrison had answered an important question, but he had raised a dozen more. Shortly afterwards a French scientist (Alexis Carrel, 1911), also working in the United States, established a diet for many sorts of cells in which they could function, grow and multiply indefinitely. He established cells from the heart of a chicken which continued to live for more than thirty years and were only lost after his death at the end of the Second World War (Fig. 3). But the diet he used was still 'haute cuisine'—of blood serum and tissue juices. At that time vitamins and nucleic acids were as yet unknown and the constituents of proteins and enzymes only beginning to be studied. A 'synthetic' diet was quite beyond the reach of current technical knowledge.

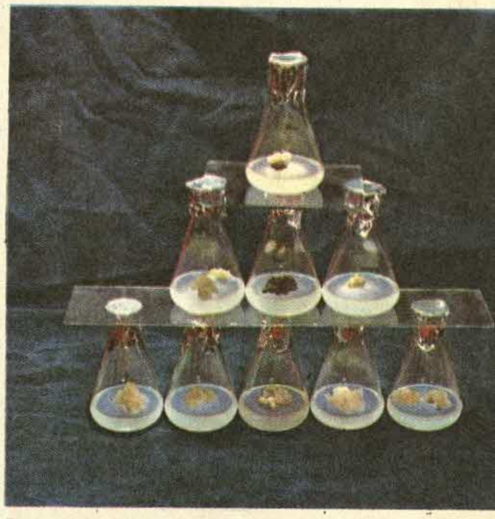
Synthetic food for plant cells

Synthetic diets, for obvious reasons, were first developed for plant cells. They could be simpler. With few exceptions plants are completely 'autotrophic', taking their food in the form of inorganic materials: water, air, the salts of the soil. From these they build all of their constituents. They build carbohydrates by utilising the sun's energy to join the carbon dioxide of the air and water from the soil in the process which we call 'photosynthesis'. These carbohydrates are then oxidised to provide energy for synthesis of proteins, for fixing the high-energy phosphate bonds and the nitrogen essential to living matter. Fats are made by the union of carbon and hydrogen at still higher energy levels, and the complex vitamins and hormones arise by incorporation of the catalytic functions of the heavy metals; iron, manganese, copper, zinc, molybdenum, cobalt and probably others. These processes all go to show the high synthetic capacities, the high degree of independence of plants. The cells of plants, like the organisms themselves, are relatively autotrophic and can grow and function in quite simple nutrients. Not all cells are equally independent; not all possess the machinery for photosynthesis. In growing isolated plant cells we must usually provide a sugar—dextrose or sucrose. Many or perhaps most cells lack the mechanisms for making certain vitamins; these too must be supplied. In fact one, thiamin, must be supplied to all cells of which we have as yet any direct knowledge; we do not yet know *where* in the plant it is synthesized, although it is probably by some form of photosynthesis, since some tissues, such as carrot root, will make their own thiamin if they are kept in the light.

Nor can all cells, or even most, synthesize proteins from inorganic elements without help. Some will build protein from nitrate *if* they are supplied with the vitamin, pyridoxine. If they do not have pyridoxine they must be fed a simple nitrogenous compound; glycine. And if they are given neither pyridoxine nor glycine we must start still higher in the scale of complexity, supplying a series of amino-acids to be coupled together to form proteins. Nevertheless, those requirements are fairly simple and clean-cut. In no case do we need to supply plant cells with more than about a score of substances, and all of these are stable compounds of molecular weight less than 500.

The knowledge on which this statement is based has accumulated gradually over the

Fig. 1. Cultures of a variety of plant tissues grown on a synthetic nutrient of the same formula as that used for the tomato roots shown in fig. 4.



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1946
P. R. WHITE maintained animal cells at significant functional levels in a completely defined nutrient for long periods, but without growth.

1954
P. R. WHITE obtained rapid, continuous growth of animal cells in nutrients defined except for 1 per cent of serum.

1956
C. WAYMOUTH replaced the 1 per cent of serum in White's nutrient with an equal quantity of purified peptone of relatively simple structure.

1959
C. WAYMOUTH demonstrated continuous, rapid growth of animal cells in completely defined, stable, inexpensive and easily prepared nutrient solutions.

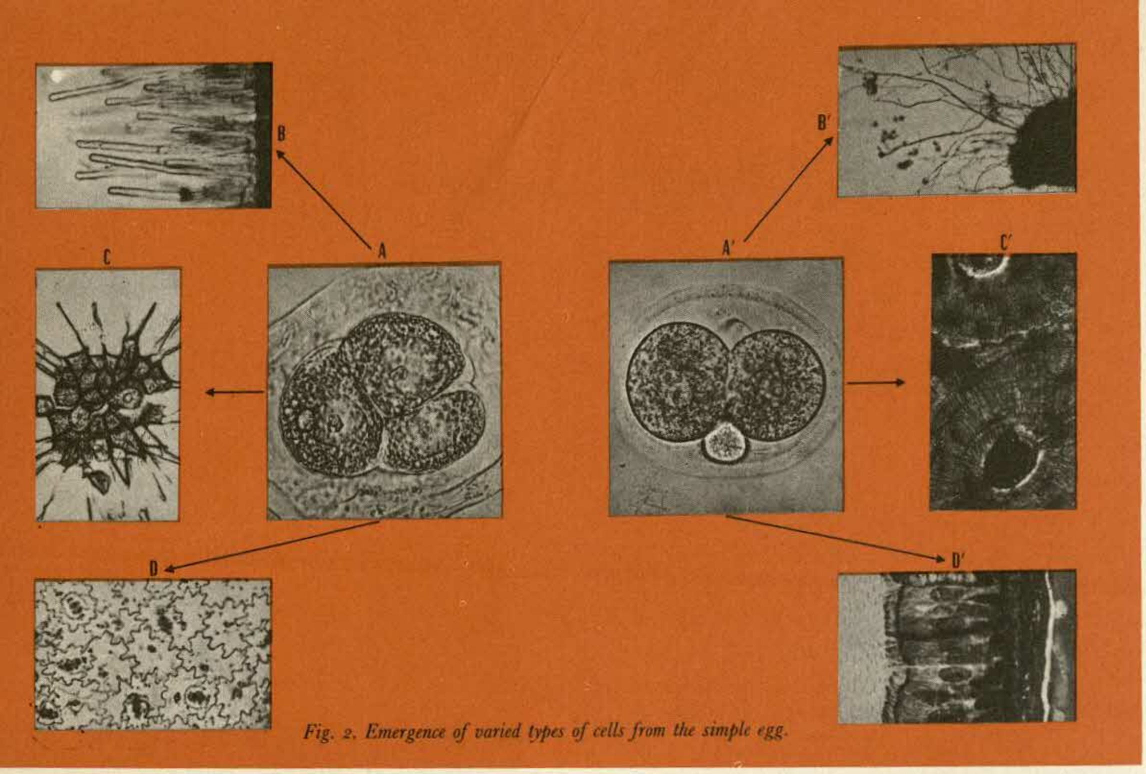

Fig. 2. Emergence of varied types of cells from the simple egg.

A. *A three-celled egg complex from the embryo-sac of the daffodil. This is before fertilisation and probably represents three equipotential cospores. Living material. (Photo Philip R. White).*

A^1 *A two-celled complex from the Rhesus monkey after fertilisation—the first division in the formation of the embryo. Living material. (Photo Warren H. Lewis, Carnegie Embryological Laboratories, Baltimore, U.S.A.).*

B. *Root hairs of tomato, taken from a root grown as an isolated member (tissue culture) on a defined nutrient. (Compare fig. 4). (Photo by Philip R. White).*

B^1 *Human spinal ganglion cells in culture, stained with silver. (Photo Edith Peterson, College of Physicians and Surgeons, New York City).*

C. *Stone-cells from the flesh of a pear fruit. (Preparation from Carolina Biological Supply Co., Elon College).*

C^1 *Human bone. Section. (Preparation from Carolina Biological Supply Co.).*

D. *Surface view of epidermis stripped from a leaf of* Coleus. *(Photo Philip R. White).*

D^1 *Ciliated epithelium from cat's trachea lining. Stained section. (Preparation from Carolina Biological Supply Co.).*

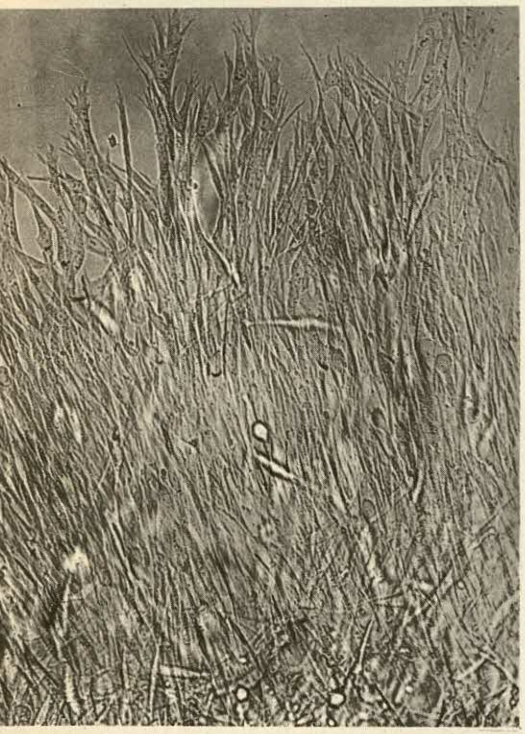

Fig. 3. Margin of a culture of Carrel's original strain of chick-heart fibroblasts after 27 years' cultivation. (From the unpublished files of A. H. Ebeling, by permission of R. C. Parker, Connaught Laboratories, University of Toronto.)

past century. Knop defined the salt requirements for plants in 1865. Haberlandt in 1902 saw the need for dividing plants into their constituent cells but did not succeed in keeping such isolated cells in an active condition. After twenty years of effort it fell to an American and a German, working independently, to show that isolated plant roots would grow for short periods in Knop's salts if a sugar and a little yeast extract (Robbins) or meat extract (Kotte) were added (1922). Later another American (White, 1934), by using a different sugar together with yeast extract, succeeded in establishing a colony of tomato roots which is still growing after thirty years in culture. The yeast extract soon yielded to analysis and proved to be supplying only two essential materials: the vitamin thiamin, and either another vitamin pyridoxine, or the amino-acid, glycine. Here, then, was a completely defined, simple diet for tomato roots (Fig. 4). It was only a simple step to growing other, less highly organised plant tissues. This was accomplished simultaneously (1939) and independently by White in the United States, by Gautheret in Paris, and by Nobécourt in Grenoble. We have now grown enough different sorts of plant tissues to convince ourselves that any tissue can be grown in defined nutrients if we devote sufficient study to learning its needs (Fig. 1).

All animals are parasites

The methods were thus available for the cultivation of isolated cells. But the problems as regards animal cells were somewhat different. Unlike plants, animals are not autotrophic; they cannot build their substance from simple elements, nor utilise directly the energy of the sun. All animals are parasites, if not on other animals at least on plants. We say this in a milder form in calling them 'heterotrophic'. And since all animals depend on the products of plants for their energy, we should expect somewhat less independence of their cells. Their basic nutritive patterns will be more complex; the levels at which the metabolic cycles must be entered will be higher levels.

While we can feed a tomato root calcium chloride, potassium nitrate, magnesium sulphate, sodium phosphate, sugar, thiamin, pyridoxine and glycine, with traces of iron, manganese, zinc, copper, molybdenum, boron and iodine, and expect it to grow, there are few if any animal cells which would not die fairly rapidly on such a diet. Although animal cells *have* been grown in profusion since Harrison's and Carrel's experiments of half a century ago, the standard diet has been that outlined by Carrel consisting of the complex materials found in serums and fresh tissue juices. It has been assumed that the synthetic capacities of animal cells are very low indeed, and that little could be accomplished without these complexes. Many studies seemed to support this view. Such notions become tradition.

Although a method of study had proved successful with plant cells, and an immense store of knowledge about the chemistry of foods had accumulated over the half century, that knowledge could be utilised for animal cells only by a sharp break with the traditional point of view. Traditions are real bonds to those who have grown up under them; they are most easily broken by outsiders. Thus it was not unnatural that the first steps should be taken by those who did not hesitate to step in where angels fear to tread. In 1946 White, utilising this accumulated knowledge and the experience gained from handling plant cells, devised a complex mixture of salts, sugar, vitamins and amino-acids in which chick tissues would live and function, though without growing, for many months, without serums or tissue juices (Fig. 5). When this mixture was substituted for the salt portion of Carrel's diet, the tissue juices could be eliminated and the serums greatly reduced without reducing the growth of cells fed thereon. Here was a diet which, with only 1 per cent of serum instead of Carrel's 60 per cent of tissue materials, would nevertheless serve all the functions of the 'haute cuisine' (Fig. 6). There quickly followed the substitution of 1 per cent of a peptone—a fairly well defined and relatively simple protein constituent—for the serum (Fig. 7), and in a few more years, by refinement of detail and without any increase in complexity, the peptone was discarded and a completely defined diet produced (Fig. 8).

Simplest diet for animal cells

This diet consists of fourteen salts, mostly the same ones used for plant-cells, a single sugar, though a different and simpler one than that used for plants, a series of vitamins, including many which are made for themselves by most plant cells, a rather large group of amino-acids, some of which will probably not be needed by all cells, and a single 'purine' (protein-building block of known chemical formula), hypoxanthine. With these forty stable, defined ingredients mouse cells of a considerable variety will grow rapidly and with great uniformity, as indefinitely as did Carrel's chick heart or White's tomato roots. Here is at least one answer to the question, 'What is the *simplest* diet on which a mammalian cell can survive, grow and multiply at a normal rate?'

This opens up vast areas for study. So far we have dealt with the problems at a generalised level: What are the requirements of *any* cell that we may be able to use? The mouse connective tissue cell was as good a one as any to start with. But we can now begin to ask more specific questions: What will a particular *type* of cell need for consummation of its particular special functions? A bone cell (Fig. 2 c^{1}) will obviously require more calcium to make good bone than will a nerve cell (Fig. 2 B^{1}) whose speciality is acetylcholine; a thyroid cell will need iodine to make thyroxine, a pancreas cell will need zinc to make insulin, a bone marrow cell will require, somewhere along its history, large quantities of iron out of which to forge the haemoglobin of the erythrocytes, a muscle cell will need more sugar and more high-energy phosphate bonds for its characteristic activity.

Actually, we *know* as yet very little about these special requirements, and nothing whatever from studies in defined nutrients. What we do know comes from the tedious and imprecise use of the older methods, but that little is enough to show what must be done. Drs Honor Fell and Mellanby, working in Britain with embryonic chick skin and using nutrients made from serums taken from

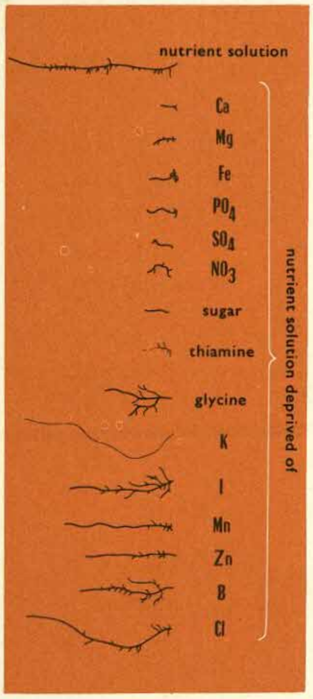

Fig. 4. Shadowgraphs of tomato roots showing the effects of omitting single ingredients from the synthetic nutrient used since 1941. The photographs were taken after five passages in the nutrients under study.

Fig. 5. A culture of embryonic chick heart muscle maintained for fifty days in a completely synthetic nutrient. The tissue has continued to beat rhythmically but there has been no growth. (Preparation by Philip R. White, 1946).

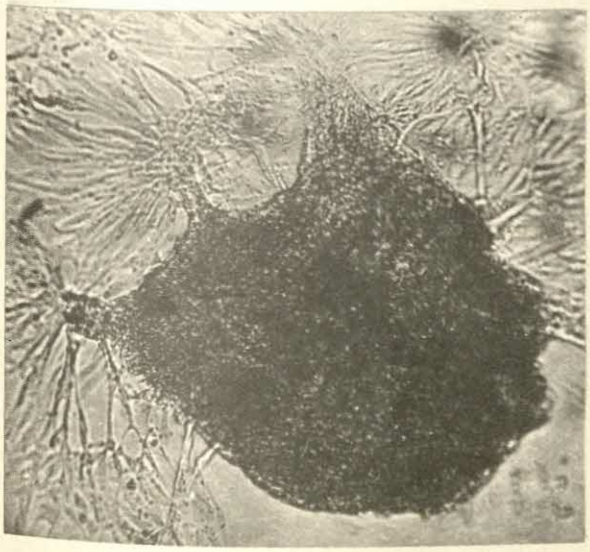

Fig. 6. Mouse fibroblasts (National Cancer Institute Strain L, Clone 929) grown for six weeks in a synthetic nutrient containing 1 per cent of rabbit serum. Note the mitotic figure. In this nutrient these cells will grow rapidly and continuously. (Preparation by Philip R. White, 1956).

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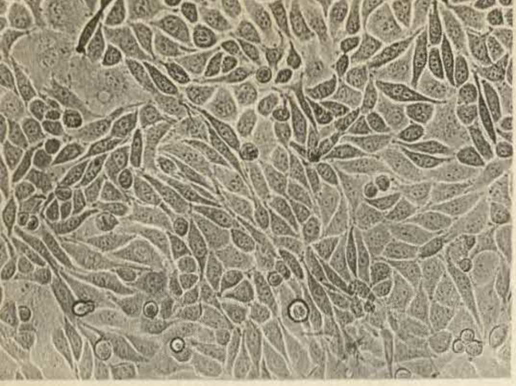


Fig. 7. Mouse fibroblasts of the same strain as fig. 6, in a similar nutrient in which the serum has been replaced by 1 per cent of a peptone. (Preparation by Charity Waymouth, 1958.)

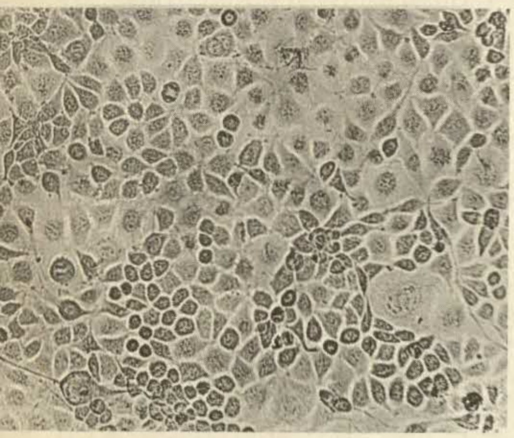


Fig. 8. Mouse fibroblasts of the same strain as figs. 6 and 7, maintained for twenty-two passages in a completely synthetic nutrient without serum, peptone or other undefined material, these being replaced by salts, amino-acids and vitamins from the chemists' shelves. (Preparation by Charity Waymouth, 1959.)

animals starved for vitamin-A or fed excessive amounts of vitamin-A, showed that epithelium could be caused to become hard and scaly like that of a bird's lower leg by withholding the vitamin from its diet, or to develop into mucous membrane or even ciliated cells like those of the lining of the lung, if provided with sufficient quantities of this one substance. This is a useful result for those of us who wish to understand the details of those maladies caused by vitamin deficiencies, and also for those interested in the more esoteric questions of the origins of particular tissue patterns in the body, the questions which we group under the rubric of 'morphogenesis'.

Dosage of nutrients

With animal tissues we have scarcely made a beginning in such studies with defined nutrients. A beginning has been made with plants. The pith from the centre of a tobacco stem is relatively inert; if it is placed on a nutrient which is 'complete' for carrot combium it will do absolutely nothing. If one growth substance, an 'Auxine', is added, there will be some enlargement, a few cell divisions, and finally some roots will develop. If another substance, kinetin, replaces the auxine the results are about the same. If *both* auxine and kinetin are added together the pith grows rapidly into a disorganised mass. If auxine is applied, then omitted and replaced by kinetin, the result is about the same as if either was applied alone; but if this sequence is reversed there is rapid growth. Evidently the kinetin prepared the ground for the effects of auxine but cannot complete the action by itself.

If instead of auxine or kinetin we apply an amino-acid, tyrosine, we get nothing, but if we apply tyrosine with the other two substances or as the third member of a sequence, we get, instead of a disorganised growth, the organisation of great numbers of stem initials with leaves.

Other materials, especially sugar and phosphate, play collateral roles in these processes. By a judicious manipulation of the relative concentrations of all these substances we can obtain either no growth at all, or stem tips alone, or roots alone, or both stems and roots, or an exuberant, formless, disorganised growth resembling a tumour. If, instead of applying auxine in the nutrient substratum on which the tissue is placed we incorporate it into some mass which can be applied unilaterally *on top* of the tissue block, the cells down the centre of the block will differentiate into typical elongated strands of conducting units, the so-called 'vascular strands'. A low concentration of auxine induces a single, compact central strand. Higher concentrations produce multiple strands, and if the concentration is high enough, these strands will form in a ring, leaving a central core of unorganised tissue, quite like the outer ring of vascular strands and inner core of pith found in a typical stem.

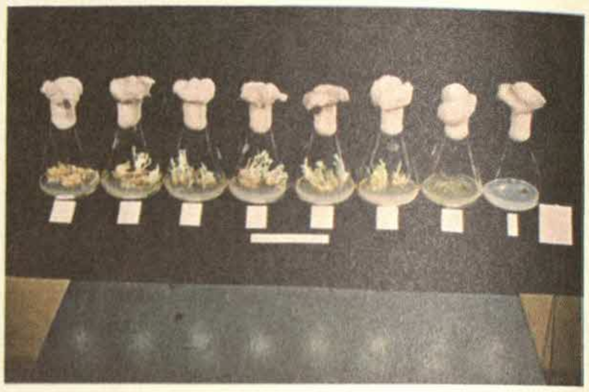


Fig. 9. Bits of tobacco pith grown in a nutrient which contains all the salts and carbohydrate necessary for survival and growth but with varied concentrations and combinations of two special essential substances, the auxine: indole-acetic acid (IAA) *and kinetin. (Published by permission of Folke Skooq, University of Wisconsin.)*

Concentrations in mg/1 from left to right

Kinetin	0	0	10	10	10	10	10	10
IAA	0	0	0	0·005	0·03	0·18	0·18	30
Casein hydrolysate	0	3000	3000	3000	3000	3000	3000	3000

We are thus able to *direct* development along pre-selected channels and to get some preliminary notion of the sort of factors which control normal morphogenesis. This is regional nutrition, a defining of the nutrition of individual units in the organism-society. We cannot yet deal with animal organisation with like precision but we do now have available the necessary methods.

We have come a long way from the 'haute cuisine' of Carrel. We have a long way yet to go before we can completely define all the important qualities of the environments of all the citizens of our organism-societies which are our fellow living creatures.

Cell mobility and tissue stability

John P. TRINKAUS

Many features of adult vertebrates distinguish them from embryos. Of these, one of the most impressive to the developmental biologist is the histological and positional stability of the cells and tissues. The more spectacular features of development are over and the various tissues and organs have been formed, each in its proper place. Each cell will now maintain its form and function and remain in its particular tissue home essentially unchanged, except for an occasional cell division, until death itself intervenes. Some kinds of tissue cells such as neurones or nerve-cells even cease to divide. Indeed, so typical and predictable is cell differentiation and so stable its cellular products that the rare changes that do occur are 'pathological'.

Cell lability in embryo and adult

We should remember the embryonic forebears of adult tissue cells are often strikingly unstable. Not only may one observe progressive changes in cell character as development proceeds, but by the simple expedient of transferring a mass of cells from one locus to another of an early embryo one may totally change the histological fate of the cells. Thus cells which would normally form spinal cord, if left in place, may be forced to form skin epidermis. From a positional point of view embryonic cells show similar lability. They may move in concert with other cells, in sheets or as an invading mass. In fact, such cell movements are so extensive at certain times in development, as during *gastrulation*,[1] that cells may move literally from one end of the embryo to the other or from the most superficial layer to a position deeply imbedded inside. But this is not by any means the only movement available. Many embryonic cells migrate great distances as individuals, through the tissue interstices, to accumulate in certain predestined areas, there to settle down and form new tissues. In this manner the *neural crest*[2] becomes dispersed throughout the embryo, later to give rise to several different types of tissue. Similarly the germ cells often find their way to the gonads from distant tissue sites. Embryonic blood-forming cells are legendary in their migratory capacities. It is apparent that for many kinds of embryonic cells extensive mobility comes as readily as cell division. But once each cell reaches its destination, migratory activity appears to cease.

This brings us to advanced stages again, this time to look for evidences of corresponding lability. When examined in this way a surprisingly large amount of instability shows up. If parts of adults are removed (especially among the lower vertebrates), complete replacement often occurs. The exact tissue to tissue source of the cells of the regenerate is not yet settled, but it is perfectly clear that during regeneration certain adult cells are capable of engaging in a number of activities that are usually the province of embryonic cells: mitosis, cell migration, loss and restoration of cell character, and probably even metaplasia.[3] Next there is the problem of cancer. For reasons still poorly understood normal tissue cells occasionally transform into cells with high mitotic and migratory activity and often the malignant capacity to invade other organs. Unfortunately, we have not the space here to discuss these histogenetic problems further, or even mention other instances in which metaplastic changes have been induced in the laboratory. We have therefore decided to limit ourselves to the locomotory features of vertebrate cells; this is where the emphasis must lie.[4]

We have already noted the possibility of extensive migrations in bizarre situations such as regeneration and cancer. More commonplace examples are the rapid spreading of sheets of epithelial cells to cover a wounded surface and the active movements of fibroblasts from a piece of cultured tissue. For migratory activity in the normally functioning organism, recall the peripatetic macrophages and lymphocytes that apparently travel at will through the tissues and play such an important role in the continual battle against foreign invaders. And there are others. One of the most strikingly mobile cells is the pigment cell of the feather germs of birds. If grafted to an embryo these cells migrate long distances to pigment developing feathers (fig. 2). Here we have unquestionable capacity of adult differentiated cells to engage in the very migratory activities characteristic of their neural crest ancestors in the embryo.

One of the most arresting features of most adult tissue cells is their apparent immobility. Is the apparent immobility of most tissue cells due to intra- or extra-cellular inhibitory factors? And why stop here? While we are asking questions we might even challenge our original generalisation. Perhaps the topo-

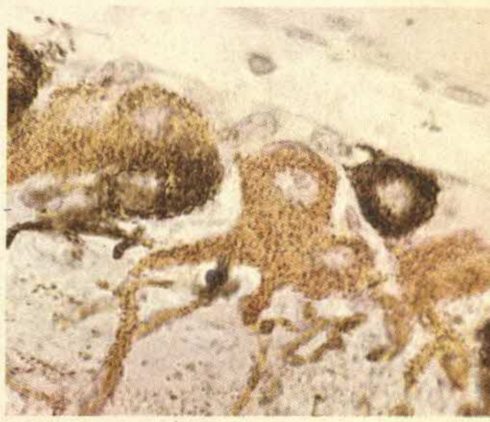


Fig. 1. Black and red pigmentary cells in the feather of an adult chicken. Note the star-shaped form (photo Trinkaus).

(1) Gastrulation: *a process of early embryonic development during which there is a complete reorganisation of the embryo by extensive cell movements; at the end of gastrulation the main organ primordia occupy the same positions as in the adult.*

(2) Neural crest: *an inconspicuous layer of cells dorsal and lateral to the embryonic brain and spinal cord, the cells of which migrate as individuals throughout the embryo to give rise to cells and tissues as different as pigment cells of the skin, spinal and sympathetic ganglia, sheath cells of nerves, cartilage of the head and neck and that part of the adrenal gland that produces epinephrine.*

(3) Metaplasia: *the transformation of a fully differentiated cell into another type of tissue cell.*

(4) *Suffice it to say that our early emphasis on the undeniable histological stability of adult tissue cells is incomplete. Metaplasia is possible and does occur. How extensive it is, how it is controlled and integrated by the organism, what significance it has for the ever-pressing problem of cancer control, are questions being asked daily by powerful new experimental methods.*

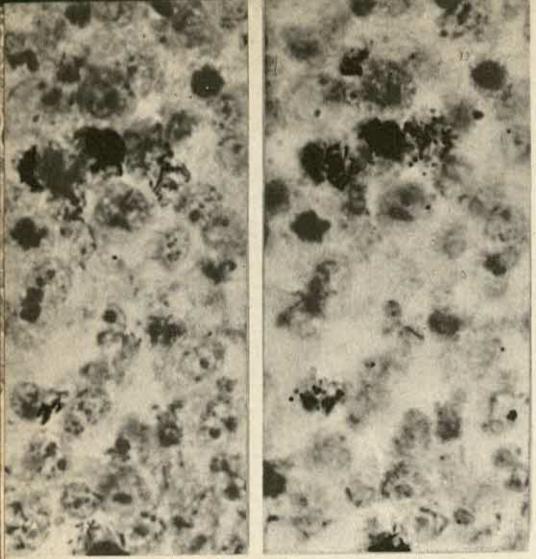


Fig. 2. A mixture of pigmentary cells from the retina of a six-day chick embryo marked with thymidine ^{3}H and non-radioactive cells from the kidney of a four and a half days chick embryo prior to culturing. Photograph A *is focussed on the cells; note the rod-shaped pigmentary granules. Photograph* B *is focussed on the photographic emulsion covering the histological section; note the black grains of silver which show up the radioactivity of three pigmentary cells. None of the kidney cells is radioactive (photo Trinkaus).*

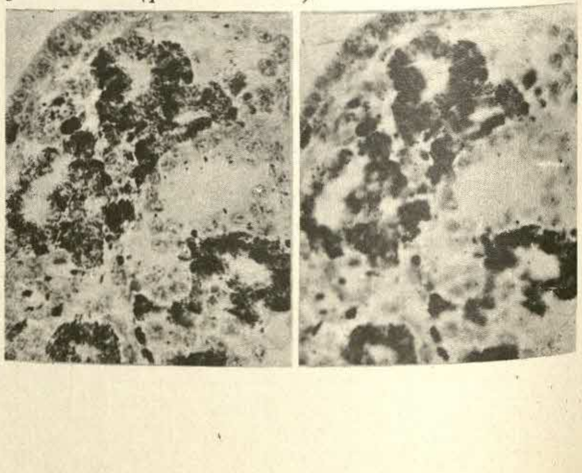
graphic stability we see in sections of adult tissue is statistical, *viz., a steady state*^{5} stability with a slow, steady turn-over or moving in and out of cells?

Methods of study

Three methods that have in the past revealed much about cell movements in the embryo are unfortunately inadequate for the problem before us. Close observation of the intact organism or of carefully staged sections of fixed organs can at best only give suggestions concerning the movements of *sheets* of cells; for following individual cells the method is of no use at all. The surgical extirpation or addition of a part, followed by a search for anatomical deficiencies or additions, methods that have been so fruitful in exposing the varied activities of the neural crest, are of little use in studying advanced stages which already possess all of the tissues. And that method with which the German embryologist, Vogt, charted in elegant fashion the movements of gastrulation, the marking of cells by dyes which do not affect their viability, is of limited use for studying the movements of individual cells. The colour is often too light to be detected in single cells, or it is reduced to a colourless state by the cell's oxidative activity. Marking cells is clearly the best method; but to be adequate a marking method must meet four requirements: (a) it must mark individual cells, clearly and distinctly, (b) it must not interfere with the normal migratory activities of cells, (c) it must remain in or attached to the cell for a long period of time, (d) it must not transfer to other cells. These are stringent requirements and not readily met. Fortunately, the biologist has had friendly helpers in his search, not the least of these being Nature herself. Certain cells are readily distinguished from others by the presence of black pigment granules. By ingenious use of pigment differences of different breeds of chickens or species of salamanders the American embryologists Willier, Rawles and Twitty have followed pigment-cell movements in both embryonic and advanced stages in great detail. We ourselves, as will be seen below, have taken advantage of the pigmented state of certain cells of the retina to follow their migrations in tissue culture. Nor has Nature's generosity stopped here. It turns out that mouse and chick cells are readily distinguishable by their different reactions to certain biological dyes (fig. 3). This, combined with the fact that embryonic cells of these widely separated species will tolerate each other in cell mixtures, has made it possible for E. Wolff and his students in Paris and an Israeli biologist, Moscona, to follow the movements and interactions of cells of several organs.

Every method has its limitations, and it is the recognition of the weaknesses as well as the strengths that permits its most effective use. Pigment granules mark only pigment cells. If one wishes to study cell behaviour of grafts at advanced stages, mouse-chick combinations are useless; immunological antagonism will destroy the graft. Other methods are therefore needed. Our aid now comes from the physicist and the biochemist. With radioactive isotopes such as carbon,^{14} sulfur,^{35} phosphorus,^{32} etc. a means is provided of marking individual cells distinctly, safely and for sufficient periods of time. The only worry has been the possibility of transfer to other cells, because of the dynamic state of cell chemistry. At this juncture nucleic acid biochemistry has come to the rescue. The DNA of the cell nucleus does not turn over; it simply reproduces itself at each cell division. If we could feed cells a labelled compound that would be combined exclusively into this new DNA, we would have an excellent cell marker. *Thymidine* labelled with *tritium* (thymidine ^{3}H)^6 appears to be just what the doctor ordered. Miss Marcie Gross and I have just completed experiments to see if there is any transfer of radioactivity to unlabelled cells. As a test system we used cells already tagged by pigment granules, labelled them with thymidine ^{3}H, mixed them with unlabelled non-pigmented cells and cultured them together for several days (figs. 4 and 5). We also did the converse experiment (figs. 6 and 7). One can see by comparison of photos before and after culturing that the thymidine ^{3}H has remained in the cells. In thymidine ^{3}H, therefore, we have a label that meets all our criteria and

Fig. 3. The same experiment as in fig. 2, after five days of culturing. Re-grouping of the cells has taken place; note that, even after five days, no transfer of radioactivity to the non-marked cells has taken place. Note the presence of renal tubules and of three pigmented tubules, demonstrating the capacity of the pigmented cells of the retina to form tubules (photo Trinkaus).



(5) Steady state: *a superficially unchanging state which in reality is completely dynamic, with parts being added and taken away continually at an equal rate. On a biochemical level this is one of the most fundamental of physiological concepts.*

(6) Thymidine *is one of the chemical building blocks of* DNA. *It is combined exclusively into the new* DNA *that is made prior to each cell division. Tritium, the radioactive isotope of hydrogen, is ideal as a cell marker. It produces emanations of such low energy that they activate silver grains in photographic emulsion for a distance of only 1 micron or so. It is therefore possible to localise the radioactivity to individual nuclei and cells. Thymidine* ^{3}H, *which has been used to study the behaviour of individual chromosomes, has a half-life of approximately twelve years.*

(7) *Incidentally, now that we can label almost any type of cell we should also be able to gain answers to many old developmental questions. Take, for*

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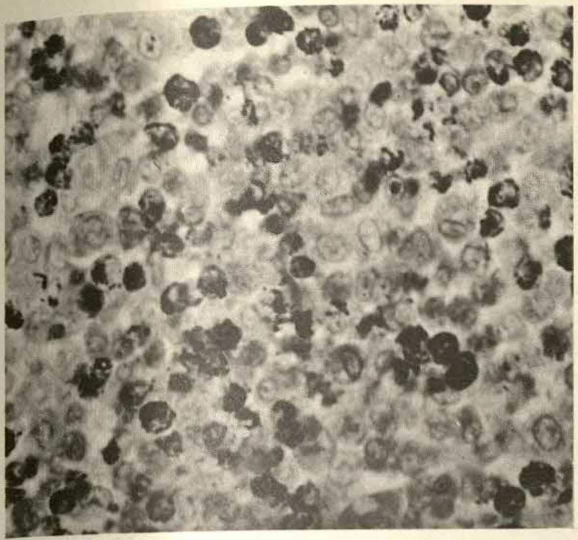

Fig. 4. A mixture of the pigmentary cells of the retina of a six-day chick embryo and the lung cells of an eight-day chick embryo, before culturing. The retina being composed of only one type of cell, we can be certain of the origin of the lung cells in the mixture (photo Trinkaus).

can be used for any cells undergoing division.

With these various marking methods at hand, with new methods of releasing cells from their tissues and with improved cell and tissue culture, these last years have seen a concerted attack on this problem of cell migration. Let us examine the results.

Migratory capacities of differentiated cells

The only way to determine whether the apparent cell immobility we see in sections of differentiated organs is real, or is merely a snapshot of a cryptic, migratory situation, is to mark certain of the cells and follow their topographic fate *in situ*. This research has hardly been started. In consequence, the actual migratory activity of differentiated cells remains unsettled and begging investigation. With long term marking methods now available, the near future should tell us whether our past assumptions have been correct or not.

As far as migratory *capacities* are concerned we are in a happier situation. There is much new evidence, and this evidence has raised so many new questions that a whole new area of biological investigation has opened. Although the work to be described has all occurred during the last decade, the basic methods and approach were developed years ago. In 1907 an American biologist, H. V. Wilson, described some remarkable experiments with sponges. He squeezed whole sponges through fine cloth into seawater, breaking them up into individual cells, small clumps of cells and fragments. When allowed to settle to the bottom of the dish the cells assembled into groups, which then merged, reorganised and reconstituted small, complete sponges.

Unfortunately this fascinating story had little effect on those studying vertebrate development. It was no doubt thought that only simple animals like sponges could withstand the punishing dissociation treatment. Moreover, during the early years of the century embryologists were preoccupied with early embryonic stages, and histologists still thought mainly in static, descriptive terms. At any rate, it was not until 1944 that reconstitution by dissociated vertebrate cells was demonstrated. The experiment was performed in Canada by Holtfreter (a German who has done much pioneering work in embryology) with the embryonic kidney of a salamander. During the same period in the United States P. Weiss and Tyler each published papers calling attention to the specificites of cell interactions and advancing theories of explanation. Soon after came news that one could obtain organ reconstitution with cells of a higher vertebrate, the chick. Moscona, working in Cambridge, showed in 1952 that *trypsin*[8] would dissociate cells of wing mesenchyme and embryonic kidney. After reaggregation these cells formed cartilage and tubules, respectively. In 1954 Zwilling discovered that an industrial *chelating*[9] *agent*, ethylenediamine tetra-acetic acid, would likewise dissociate cells, particularly epithelial types. These results with trypsin and EDTA found an immediate audience. For one could now readily release cells from the bonds of the tissue system, place them in new cellular and chemical environments and study their behaviour, migratory and otherwise.[10]

We next learned that the original tissues will be reformed, even when one mixes cells of different organs. Townes and Holtfreter showed this in 1955 for early amphibian germ layers, and Mrs Peggy Groves and I found it to be so for mixtures of kidney and wing-bud cells of chick embryos. But the former went an important step further. By taking advantage of natural differences in size and pigmentation of embryonic amphibian cells, they demonstrated that the various cell types sorted out within the explant. Reconstituted ectoderm, for example, was derived solely from ectoderm cells. A year or so later Moscona demonstrated reassortment in more advanced stages using Wolff's technique of mixing mouse and chick cells (fig. 8). We have recently confirmed the sorting out of chick cells with radio-isotope

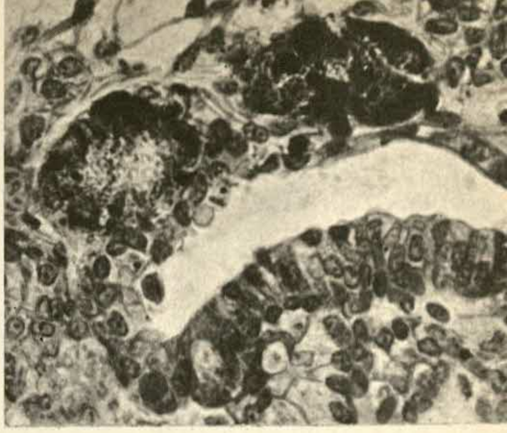

Fig. 5. Two masses of pigmented retinal cells which have regrouped in a mixture of retinal and lung cells (photo Trinkaus).

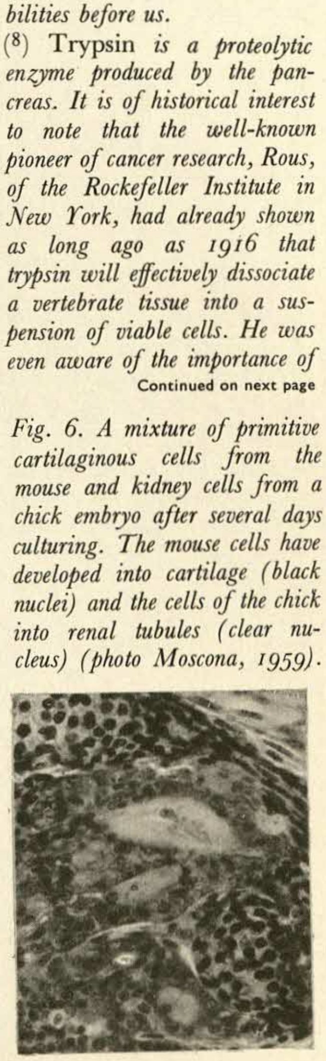

Fig. 6. A mixture of primitive cartilaginous cells from the mouse and kidney cells from a chick embryo after several days culturing. The mouse cells have developed into cartilage (black nuclei) and the cells of the chick into renal tubules (clear nucleus) (photo Moscona, 1959).

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example, the neural crest. We know very well to which tissues it gives rise, but very little about the routes travelled by the cells, and whether they migrate at random, with many missing their proper destinations, or in a directed fashion, efficiently to the target organs. This one example illustrates the provocative possibilities before us.

(8) Trypsin *is a proteolytic enzyme produced by the pancreas. It is of historical interest to note that the well-known pioneer of cancer research, Rous, of the Rockefeller Institute in New York, had already shown as long ago as 1916 that trypsin will effectively dissociate a vertebrate tissue into a suspension of viable cells. He was even aware of the importance of*

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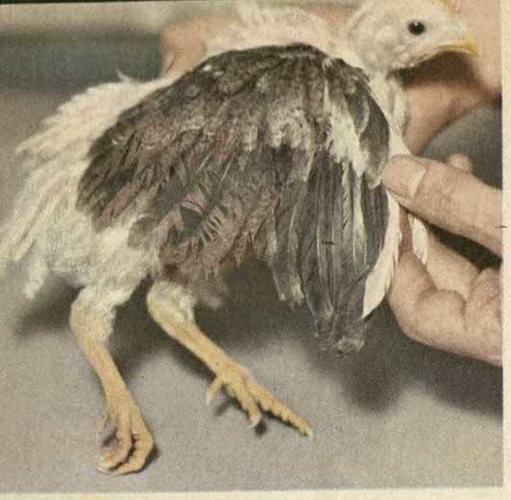


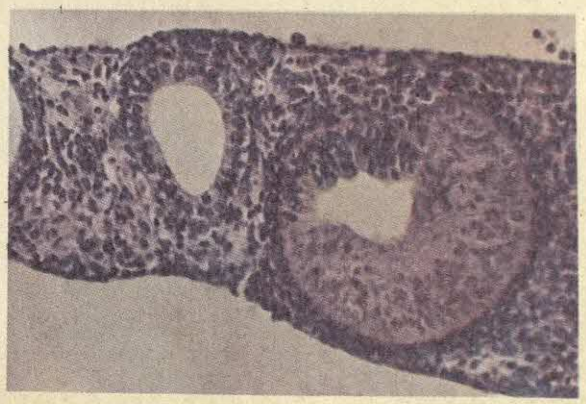
Fig. 7. Leghorn white chicken. During the third day of incubation of the embryo, this chicken was given a graft from an adult bird of the brown Leghorn breed. The pigmentary cells of the adult have migrated to the epidermis of the wing, penetrated into the wing buds, and have finally succeeded in pigmenting almost all the feathers of this wing. The pigmentary cells have adopted the pattern characteristic of the breed of the donor (photo Trinkaus).

labels S^{35} and thymidine ^{3}H. Evidently, when one dissociates vertebrate cells and intermingles them with other tissue cells they migrate within the cellular mass, find like cells, adhere to them and together with them reform the original tissue patterns.

But how does this occur? In an effort to learn something of this we mixed *retinal pigment cells* with cells of several different organs (fig. 9). The abundant pigment granules in the cytoplasm made changes in cell form readily evident. They migrated at random within the aggregate, forming small clusters. Migratory activity apparently does not cease with the appearance of small clusters, however, for often after another day or so all pigment cells are found in fewer, larger clusters (fig. 10). During the migratory period, when surrounded by foreign cells, an extraordinary change occurred. These pigment cells, which *in vivo* are cuboidal and tightly joined to each other in a typical epithelial membrane, become elongate and dendritic (fig. 11) (like migratory pigment cells of feather germs, fig. 1). After the migratory period, when clustered with other pigment cells, they resumed a more epithelioid shape (figs. 5, 6 and 7). It appears that a change to a dendritic form is necessary to enable a pigment cell to squeeze between other cells. It would be interesting to know if this is a general characteristic of cells moving through tissues.

Let us return now to our original questions. The work just outlined is obviously only a beginning, but already we are being led toward certain conclusions. All cells thus far tested are highly dynamic creatures with clear migratory capacities. They include such a variety of tissue types and stages of differentiation that it seems likely that most differentiated cells have the capacity for migratory movements. Whether or not this capacity finds expression depends on the environment. We have not answered our next problem, but we have moved toward its solution. We now know that the apparent *in situ* immobility is not due to an intrinsic lack of ability to move. It is caused by *ex*trinsic inhibitory controls. But what is the nature of these controls?

A mixture of embryonic cells of mouse (blue nuclei) and chick (purple nuclei). Although of distant genetic origins these cells have co-operated to make a single tube. (Photo E. Wolff)



Tissue and cell affinities

Again we must consult the lowly amphibian. In 1939 Holtfreter attempted to fuse intact germ layers in culture. He soon discovered, however, that it takes more than mere stickiness to make the layers adhere. It is a highly specific affair. Ectoderm and endoderm both fused with mesoderm, but rejected one another. Hence if one wishes to construct something out of ectoderm and endoderm, one must add mesoderm, else they will fall apart. Holtfreter called this recognition phenomenon *affinity*. More recently Chiakulas, working in Weiss' laboratory, showed that the same selectivity characterises fully differentiated tissues. With elegant simplicity he removed an area of salamander skin and grafted various epithelia in its place. A graft of skin spread over the denuded surface, fused readily with the host skin and ceased migration. Tissues normally contiguous with epidermis, such as cornea and oral epithelium, also fused, but not non-contiguous tissues (oesophagus, small intestine, bladder, etc.). By ringing a rod of cartilage with two tissues and thrusting the whole assemblage into the gelatinous interior of the dorsal fin, he again found fusion only between pieces of the same tissue or between normally contiguous tissues, e.g., oesophagus and lung or oesophagus and oral epithelium, but not between non-contiguous tissues such as lung and oral epithelium. Obviously, both early and advanced tissues have remarkable faculties of recognition. They can not only distinguish their own kind, but have the additional talent of recognising some of the very tissues with which they must normally associate.[11]

The reassortment that occurs in mixed cell aggregates tells us that this recognition process extends to the cell level. Individual cells possess affinity. The re-establishment of their own tissue arrangements indicates in addition that they possess a high degree of 'cell memory'. This memory is so steadfast in many cases that it is not even shaken by the proximity of entirely different histogenetic activities (fig. 8; but note figs. 5 and 7 for an exception to this). We now have the following picture of sorting out. During the first hours after culturing there are non-specific adhesions, enough to make a compact aggregate. But this

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his discovery. This seed of the future fell on barren ground, however, and the procedure had to be worked out anew thirty-six years later. Incidentally, Holtfreter used alkali for his dissociations.

(9) Chelating agent *is a compound that powerfully binds divalent and trivalent cations. The binding of calcium and magnesium is no doubt the reason for the excellent dissociating properties of* EDTA. *The term chelating agent takes its origin from its figurative resemblance to the grasping chelae of a lobster.*

(10) *The conceptual state of developmental biology at mid-century demanded these techniques and found them. And, as we shall see, the techniques themselves, by revealing previously unsuspected phenomena, have in turn raised new questions on their own.*

(11) *The existence of these affinities for contiguous tissues is a reminder that even more spectacular cross-tissue affinities exist* in vivo*: Schwann sheath cells for nerve fibres, pigment cells for feather and hair epidermis, and germ cells for gonads. In these hierarchies of affinity we no*

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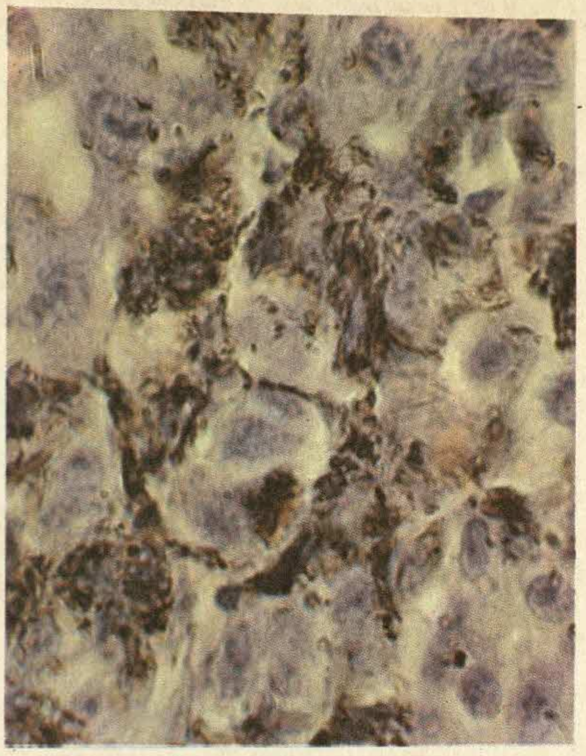
is apparently a steady state adhesion, for random cell movements continue unabated. Then, when cell A meets another cell A their type specific affinity causes them to adhere more firmly than to other cells. Small clusters form. Nearby clusters may fuse. Small clusters may break up, the cells continuing migration until they encounter larger clusters. In this fashion most cells of A type will eventually be gathered into a few large groups. Sorting out is complete and migration will presumably cease. Thus, the assumption of type-specific cell affinities seems sufficient to explain the cessation of migration.

We may now return to the organism. Why don't differentiated cells move about *in vivo*? We don't know; but we can make a good guess. They are bound to each other by their affinities. As is usual, however, each new answer raises a host of new questions.[12] Is affinity really *tissue* specific? The experiments of Holtfreter and Chiakulas and the retinal pigment cells answer 'Yes'. If affinity is tissue specific, is it an all-or-none process? Apparently not. We already know of the non-specific adhering of cells when aggregates initially form, and of affinity for contiguous tissues. Bermann, working in E. Wolff's laboratory, has recently found another example. Kidney and testis cells occasionally co-operate to form common epithelial tubules. We have similarly found a small number of chimaeric tubules composed of kidney and retinal pigment cells. Apparently any given cell has high affinity for like cells and lesser affinities for certain other cells.[11] Affinity is highly specific, but the specificity is relative. It is certainly not an all-or-none phenomenon.

Nature of affinity interactions

During this same period other laboratories, such as that of Abercrombie in London, have been struggling with other aspects of cell contacts. Their method has been to make detailed observations of the behaviour of living fibroblasts as they glide out over the glass in a tissue culture, by the use of phase and interference microscopy, time-lapse cinemicrography and careful analysis.

When fibroblasts move out on the glass there is a delicate ruffling of the thin cytoplasmic fan of the leading edge. When this cell makes contact with another cell it appears to fuse with it, and the ruffling stops at the surface of contact. Cell movement ceases. That this is primarily a surface reaction is indicated by the local nature of the inhibition and by the fact that if some surface is still exposed a new fan and ruffle will soon develop there and the cell may break away again. This means that a fibroblast is at a standstill when the only surface available is that of its neighbour cells. Conversely, as long as there is some cell-free space a fibroblast will move into it, as in the zone of outgrowth of a tissue culture.



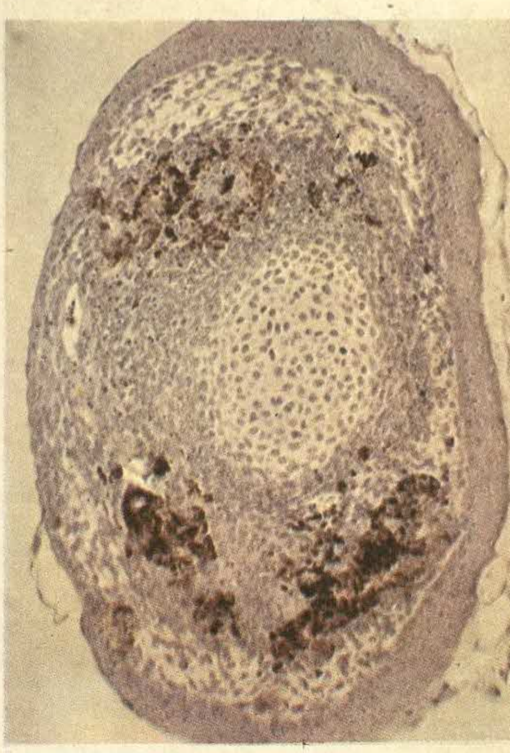


Fig. 9. Star-shaped pigmentary cells of the retina during the phase of migration after two days in culture. Compare this with fig. 1 (photo Trinkaus).

Fig. 10. A mixture of retinal pigmentary cells with the cells from a wing bud of a chick embryo after several days in culture; the regional re-grouping characteristic of this combination can be observed. Note, too, passing from the periphery to the interior, the epidermis, the loose mesenchyme, the dense perichondrial tissue which contains all the pigmented cells, and, finally, in the centre, the hyaline cartilage. The cells of the rudimentary wing have taken up the positions typical of a normal wing (photo Trinkaus).

It looks as if this 'contact inhibition' may provide a mechanism for the cessation of cell movement in aggregates. But objection may be raised that the experimental conditions in the two types of study are so different as to render comparison illegitimate. In these experiments the cells move on glass. In aggregates, cells move over other cells. It would therefore be helpful to have a control, to know, for example, whether cells which move readily over cells, both *in vivo* and in cell aggregates, will likewise move over fibroblasts. There is just such a test: invasive cancer cells are *not* contact-inhibited by fibroblasts in Abercrombie's cultures. This is a reassuring result. Certain other difficulties remain, however. The high degree of specificity, which is the hall mark of the affinity reaction, appears to be lacking in contact inhibition. Fibroblasts of skeletal muscle inhibit the movement of heart fibroblasts. How can we explain this? And what about cell mobility in beginning aggregates?

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doubt have a means for holding tissues together. They therefore have unquestionable importance in the genesis and preservation of adult organ architecture, and in preparation for emergency repair in wound healing and regeneration.

(12) *It has perhaps become apparent to the reader that in this discussion we are in fact more interested in raising new questions than in answering old ones. Many of these questions were not thought of until after the results of the experiments just described. This is yet another illustration of how very important new techniques are in a still largely empirical science such as biology. There are some who impatiently await the day when biology will have the powers of prediction and analysis possessed by the more basic and less complex fields of physics and chemistry. This day will come, no doubt. But for the present, it is the practical man in the laboratory (and in the field) that is providing most of the advances.*

If cells are in contact (and they surely are), and if contact inhibition is non-specific, why does movement continue? Although these difficulties and others are unresolved at present, they are no doubt resolvable.

On the basis of the contact inhibition theory, we assume affinity to be a character of the cell surface, and the affinity reaction to be a reaction between cell surfaces. It results in an immediate inhibition of the marginal ruffling responsible for cell movement; the surfaces adhere and movement ceases. But the inhibition is quantitative and lasts only if a large part of each cell surface is in contact. The extent and firmness of contact is influenced by a number of factors, two of the most important being surface specificity and cell population density. This would explain why cells sort out in clusters and yet do not cease migratory activity with the formation of small clusters; cells at the periphery may secondarily break loose.

It seems that whichever way we turn we come up against the cell surface.

When explanations of specific surface interactions were sought it was natural that the first thoughts should be along immunochemical lines. Immunochemistry teaches us that each tissue cell possesses type specific combining groups at its surface. If cells are immersed in antisera, those antibodies which are specific for these combining groups will be tightly bound to the cell surface. Here is a fine model for affinity. One need only postulate that cells of each tissue type possess a particular pattern of complementary combining groups; they will then be bound together by lock-key mechanisms. This theory, which was independently proposed by Weiss and by Tyler, has received support from two widely differing organisms. Spiegel discovered that re-aggregation of sponge cells can be prevented by bathing in antisera, and Gregg found the same for aggregating amoebae of a slime mould.

A related theory was given complete exposition a few years ago. This 'calcium-bridge' theory is based on the fact that cells require calcium for adhesion. Steinberg, its American promotor, reasons that surfaces of like cells have like patterns of ca-binding groups. By forming bridges between these groups ca links the two surfaces. This has been an attractive theory. It is simple; it explains the need for ca; it is amenable to testing.

If this article had been written a few months ago we would end it here. Today, however, we can move further. In February, 1960, a young British biologist, Curtis, proposed a complete rejection of both these theories. He pointed out that most cells normally have a negative charge. This would cause them to repel each other. In fact, the more alike the cells, the stronger the repulsion. Why then do like cells stick together? Drawing on the characteristics of colloid monolayers, he suggests that repelling electrical forces are opposed by attracting forces called van der Waals-London forces. The more alike the surfaces, the more powerful these forces. One may predict that the two forces will be in balance and the surfaces adhere quite firmly at a distance of 100Å–200Å. The electron microscope tells us that the separation of adhering cells is within this range. The previous theories require the cell surfaces to be *less than* 10Å apart. But what about Calcium? Curtis points out that it could increase adhesion merely by decreasing surface charge. The antibody blocking experiments could simply have covered up those groups collectively responsible for the attracting forces.

Drawing again on the characteristics of colloidal monolayers, the theory can also explain contact inhibition. Any expansion of a cell surface will increase adhesivity. When a cell moves over a substratum the *shearing* force will do just this. When the substratum is another cell the adhesivity of each will increase; the increased adhesivity of both surfaces stops movement. Sarcoma cells are assumed to have so little increase in adhesivity with shear that they do not adhere. Dissociation is thought to cause the cell surface to become more compact. Thus there will be less adhesivity with shear, until the cells have recovered. This explains the initial lack of contact inhibition in cell aggregates. Later, when the surface expands and shear promotes adhesion, the forces attracting like cells will favour adhesion at the proper surfaces. The result—sorting out.

This is a pretty theory. But theory it is, and as such requires verification.

There is one result that even a theory as ingenious as this is hard pressed to explain. In aggregates of amphibian cells ectoderm moves inexorably to the surface and mesoderm inwards. In aggregates of chick-wing bud and retinal pigment cells similar regionalisation occurs (fig. 12). Random cell movements plus affinity cannot explain this. No evidence worth mention favours attraction by diffusible chemical substances. Curtis proposes the attainment of contact inhibition by different cells at different times, such that the first cells accumulate at the surface, and so on. Perhaps.

HISTORICAL NOTES

1907
H. V. WILSON passed sponges through a sieve in sea water and found that the isolated cells reassemble and reconstitute normal little sponges.

1944
J. HOLTFRETER made an analogous experiment with the embryonic kidney of a newt.

1944
P. WEISS and A. TYLER published papers on the specificity of cellular interaction.

1952
A. A. MOSCONA showed that trypsin has the property of dissociating embryonic cells of the wing and kidney of the chick, and that these cells after aggregating together again differentiate into cartilage and tubules respectively.

1954
E. ZWILLING discovered that ethylenediamine tetra-acetic acid (EDTA) has the property of dissociating epithelial cells.

1955
P. L. TOWNES and J. HOLTFRETER proved in amphibian embryos that mixtures of cells from different rudiments are capable of reconstituting those organs. P. GROVES and J. P. TRINKAUS demonstrated the same properties in the embryo chick.

1956–1959
P. L. TOWNES and J. HOLTFRETER showed that the various types of cells regroup themselves in an explant. A. A. MOSCONA demonstrated the regrouping of cells of more advanced embryos of chick and mouse. M. GROSS and J. P. TRINKAUS marked embryonic chick cells with sulphur 35 and thymidine 3H and confirmed that cells of vertebrates dissociated and mixed in an explant migrate to the centre (where like finds and adheres to like) and reconstitute the original tissues.

1960
A. S. G. CURTIS put forward his theory of positive and negative charges on cells.

What is an organ?

Philippe SENGEL

In previous chapters, we have seen that all living things are composed of cells and tissues. We now know how cells live and multiply, and how millions of them join together to form tissues with definite features and functions.

In animals there are two main types of tissue: epithelial and connective. In the former, the cells are crowded closely together and are therefore in intimate contact with each other; in the latter, on the other hand, the cells are widely separated and only connected to each other by fine strands of protoplasm.

Epithelia may be regarded as the higher tissues of the body, for they carry out the most important functions: the retinal epithelium of the eye is responsible for the perception of light, the vesicular epithelium of the thyroid gland secretes thyroid hormone, and the epithelium of the skin (the epidermis) protects the rest of the body from injury by virtue of its remarkable strength and elasticity. But epithelia could not fulfil their functions without the help of connective tissues; for they often have little or no blood supply of their own and many of them are fragile and lack rigidity. For these reasons, epithelia are associated in the body with connective tissues which protect, support, and nourish them. This structural and functional association makes what we call an *organ*.

In the eye (fig. 1), the retina is nourished by means of the choroid, a connective tissue that is very rich in blood vessels; a second connective tissue layer, the sclerotic, which is firm, tough and elastic, forms the protective coat of the eyeball. In the thyroid gland (fig. 2) the secreting epithelial follicles are surrounded by a matrix of connective tissue cells. The epidermis of the skin (fig. 3) is intimately bound to the dermis, a connective tissue which lies beneath it and from which it draws its nourishment.

Certain organs, however, do not show this composite epithelial and connective tissue structure, but consist of a single highly differentiated tissue. An example is the neural tube—brain and spinal cord—which is wholly epithelial in origin. For its protection and nourishment, vertebrate animals have the meninges, specialised organs which envelop the central nervous system. Other organs, in contrast, are composed entirely of connective tissues, which originate from the mesenchyme of the embryo. Examples are the bones and cartilages which form the skeleton, whose cells are embedded in a strong unyielding ground-substance.

Our present-day knowledge of the origin, structure, and function of organs owes much to the science of experimental embryology. One of the most fruitful methods in this field is the technique of cultivating organs outside the body, thus enabling them to be studied in isolation from the complex animals of which they normally form part.

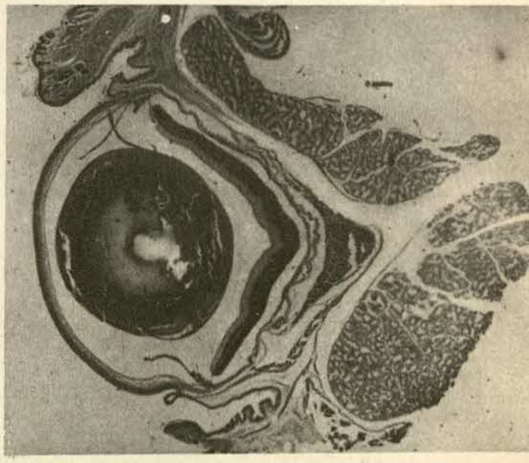

Fig. 1. Section of an eye. A section across the whole eye of a mammal (the mouse) provides a good example of the collaboration of various tissues; the retinal epithelium; the connective tissue of the choroid; and the sclerotic coat, the second connective tissue layer which forms the tough and elastic protective covering of the eyeball (mag. × 20) (photo Lod).

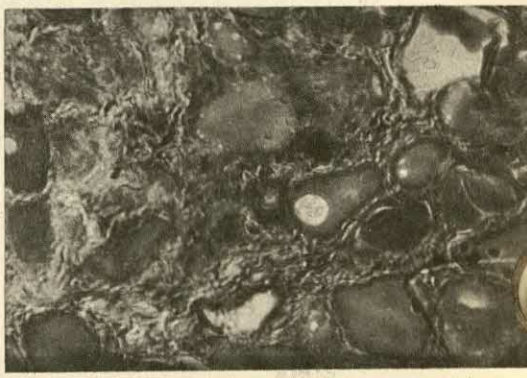

Fig. 2. Section of thyroid gland, showing the secretory vesicles surrounded by a protective covering of connective tissue cells (mag. × 200) (photo Lod).

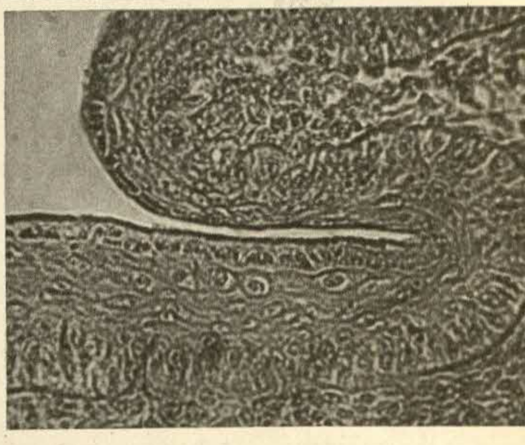

Fig. 3. Section of skin. The microscopic section shows the superficial layer, the epidermis, plainly distinct from the connective tissue dermis, which supports and nourishes it (mag. × 150) (photo Lod).

The culture of isolated organs

Emilienne WOLFF

Photographs of organs being cultivated, show experiments in the embryological laboratory of the Collège de France and the Centre National de la Recherche Scientifique.

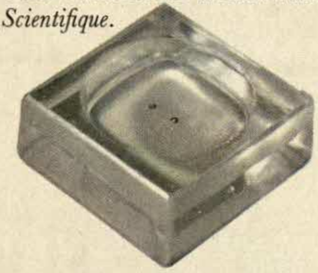

Fig. 1. Vessel in which excised organs are cultivated. This is made of glass, and contains the nutritive jelly on which the organ is placed. A square glass plate covers the vessel, and its edges are sealed with paraffin wax.

The first attempts to keep alive parts of the body after they had been removed from the organism date back to the second half of last century. Since then, research has divided into two main streams: cell culture (also incorrectly called tissue culture) and organ culture. Methods of keeping parts of the body alive *in vitro* merit the term 'culture' only if the isolated part not only survives, but also grows and develops.

Such investigations are understandably interesting from various points of view, for they may be used to find out about the nutrition of the cell or tissue, its reaction to physical and chemical factors, and even the effect of combining together different tissues and organs. These methods simplify experimental conditions considerably by excluding the control the whole organism exerts on its component parts.

Cell culture allows isolated cells to proliferate indefinitely, while free from participating in an organised structure, and is chiefly suited for studying the properties of cells. Organ culture, on the other hand, sets out to maintain the organisation of a tissue or organ, and is applied particularly to parts which have not yet completed their development, that is to say, to embryonic organs. However, before dealing with this subject, we ought to mention the various ways of isolating organs, which although they are not, strictly speaking, culture methods, are undeniably very interesting.

It is a well-known fact that the heart of a frog or a chicken embryo continues to beat if it is placed in the appropriate saline solution, that is, one which contains approximately the same percentage of salts as the blood serum of these animals. It can thus be kept alive for several hours. Furthermore, the rate of the chicken embryo's heart-beat can be slowed down or speeded up at will by cooling or warming the fluid in which it is bathed.

Perfusion and artificial heart-lung machines

An important advance was the development of the method of irrigating a detached organ through its normal circulatory ducts, such as the main vein of the heart or the arteries of other organs. This technique is known as perfusion. In this way different substances can be tested for their effect on the functioning of an organ, such as the muscular contractions of the heart, and this procedure is extensively used in physiology. One of the earliest and most brilliant examples of its use was the experiment carried out on the liver by Claude Bernard, who showed that this organ produces and secretes glucose at the expense of its reserve of glycogen. Perfusion can be improved by operating under aseptic conditions and thus preventing the organ from becoming rapidly infected. This brings us to another application of perfusion: the artificial heart-lung machine.

This machine consists of a pump whose rhythmic movements simulate the contractions of the heart and an apparatus which oxygenates the blood as the lung ordinarily does.[1] The blood, or other nutritive fluid, is passed into the organ through a flexible tube which functions as an artery and is joined to one of the organ's larger blood vessels. It is subsequently recovered from the efferent vessels of the organ and sent to the artificial lung. This is a sort of tank where the blood is spread over a large surface or plate and brought into contact with a current of oxygen. Thus regenerated, the blood returns to the artificial cardiac pump[1] (fig. 5).

These complicated machines have not been used much in embryology because they are not suitable for minute organs of embryos. Organs, such as the thyroid gland cultured by Carrel and Lindbergh were removed from old embryos. They were fairly large and already differentiated, so that these experiments served to elucidate physiological rather than embryological problems. The same applies to the foetal calves weighing several pounds kept alive for a number of days by J. A. Thomas.[2]

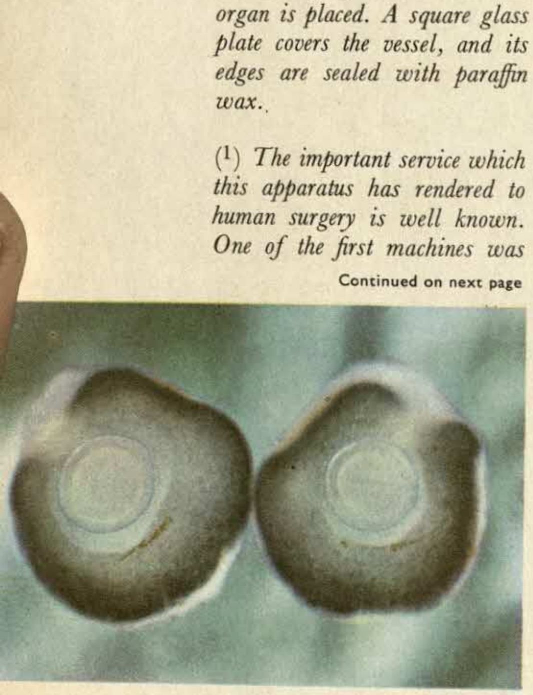

Fig. 2. Two eyes of a chicken embryo, removed on the second day of incubation, and grown for two days on a nutrient medium. The ring-shaped iris can be seen developing, and at the same time the retina is being differentiated (mag. × 50) (photo Lod).

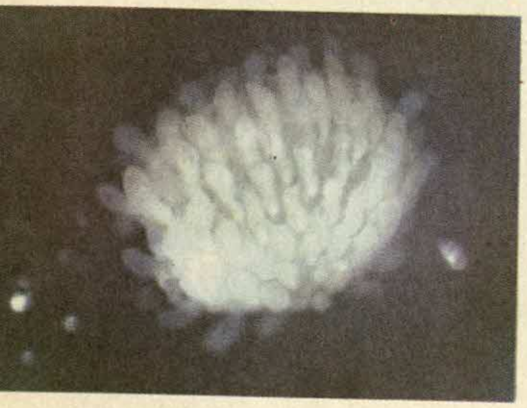

Fig. 3. Skin from the back of a chicken embryo, removed on the sixth day of incubation, when no rudiments of feathers were visible. After cultivation for ten days in a medium containing brain-extract, feather-buds are developing (mag. × 20) (photo Lod).

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(1) *The important service which this apparatus has rendered to human surgery is well known. One of the first machines was*

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Culture in vitro

The culture of embryonic organs answers quite different questions. Is it possible to keep a delicate rudimentary organ, that has not completed, or even begun, its development, alive outside the body? Can it be made to grow and differentiate just as it would have done if it had remained inside the animal? Alternatively, is it possible in an altered environment so to inhibit the organ's growth or change its fate by diverting it from its normal course of development? What are the factors necessary for normal growth and what are the ones which modify it?

The answers to these questions have been provided by modern techniques, which have made the following remarkable achievement possible: a minute organ may be removed from a young embryo, grown *in vitro* for some time, and then grafted onto an older embryo or a newly-born animal. So although we are not yet able to replace organs, we are getting remarkably close to it.

It will be seen that the techniques of culturing embryonic organs *in vitro* are based on quite different principles from those involved in keeping large organs alive for a very limited time by means of perfusion techniques or heart-lung machines.

In practice, rudimentary organs removed from an embryo are usually thin enough for atmospheric air to diffuse right through them, without any danger of the tissues being asphyxiated. They may therefore be kept on the surface of a suitable nutritive medium in a saturated damp atmosphere (figs. 1, 2 and 3) where the organ can breathe air through its upper surface without any risk of drying up, and draw its nourishment from the medium on which it rests by osmosis. Amongst those who helped to devise these methods were H. B. Fell[3] and Etienne Wolff and his team.

The medium recommended by Etienne Wolff consists of two parts. First, a non-nutritive base of agar jelly[4] made up with normal saline and secondly, a nutritive preparation which can be varied according to the problems it is proposed to study. The semi-solid agar base keeps the organ on the surface between the air and the nutritive medium. One advantage of this method is that the latter can be made up from natural products such as embryonic extract or blood serum or equally well from specific chemical compounds, which we can try to substitute for the complex natural substances.

Thus cultures may be carried out on either natural or synthetic media. Both make it possible to solve many problems, especially those relating to developmental factors, and by using synthetic media the nutritional needs of organs may be analysed.

Using the same base, the nutrient medium is prepared from a dilute extract of chicken embryo, which is adequate for the development of many organs. The fact that such media are relatively poor in nutrients is beneficial for organ culture (fig. 4), because media that are very rich in nutrients favour the increase of some cells, such as the fibroblasts (connective tissue cells) while other cells, being less

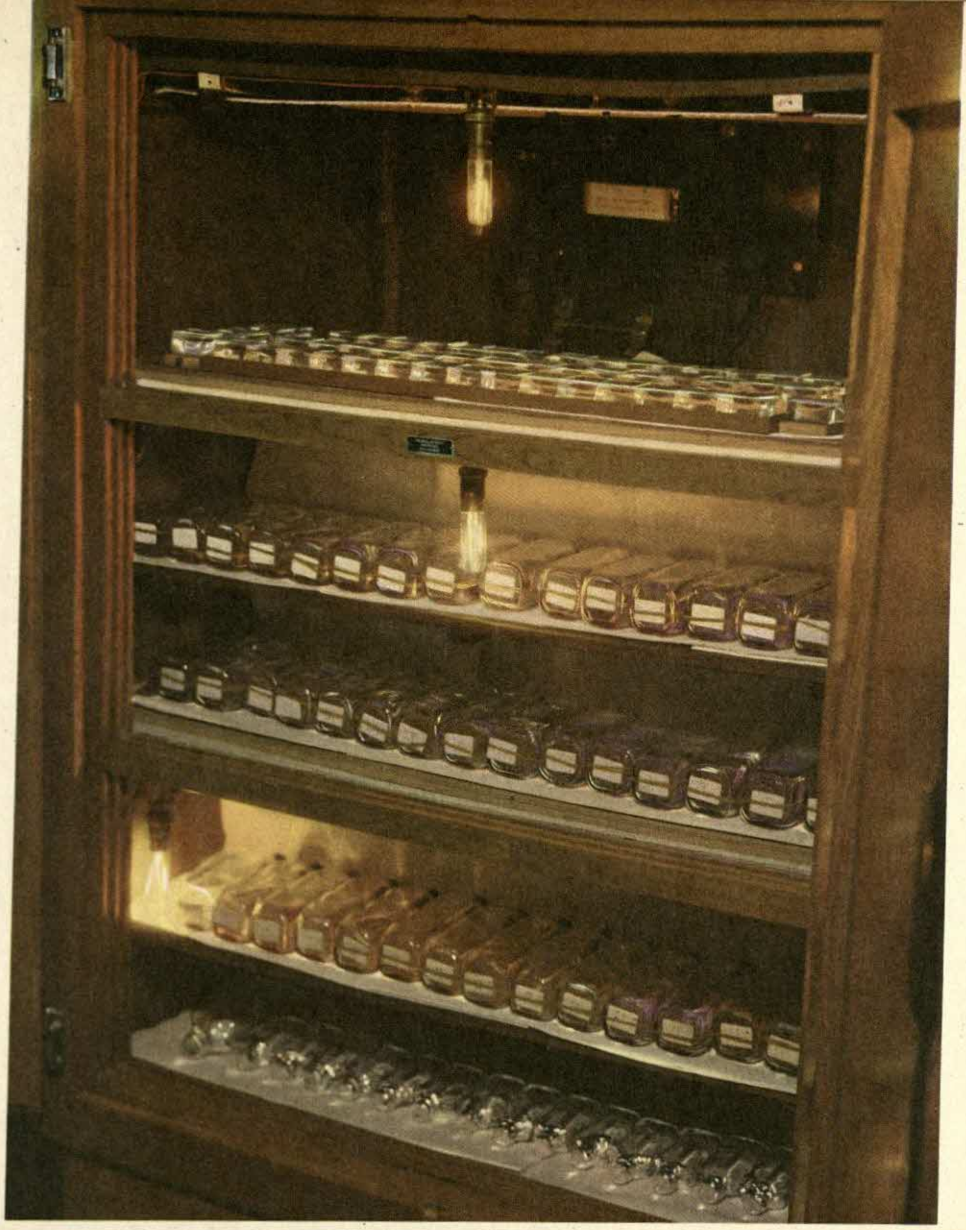

Fig. 4. Incubator in which the temperature is kept steady at about 38°c. On the top shelf are a number of vessels in which embryonic organs are growing. The other shelves hold bottles in which human cancer cells are living in culture. The cells spread out on the flat bottoms of the vessels, where they form a continuous film. They are covered by the nutrient fluid, which is coloured pink by a substance whose tint varies according to the degree of acidity or alkalinity of the medium. (Photo Lod).

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constructed by Carrel and Lindbergh who used it for organ culture, whilst one of the most recent and effective was devised by J. A. Thomas and is used currently for intracardiac operations. During the operation the human heart is completely outside the circuit.

(2) *Another spectacular physiological experiment was carried out with the aid of a heart-lung machine by Peters and Massart who removed a cow's udder aseptically, just as it was slaughtered. They connected the vessels of the mammary gland to the nozzles of the machine in which cow's blood was circulating. Milk was then seen to flow from one of the nipples of the gland. Thus milk had been 'made' from blood in a closed vessel and the organ, although*

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it was isolated from the living animal, still carried out its natural function of secreting milk. By varying the blood composition, either by adding lipids, sugars, or even water, the effects of these on the composition of the milk could be studied. Thus milk could be adulterated through the mediation of the organ itself. Injecting pituitary hormone into the rubber artery which connected the cardiac pump to the organ brought about an increased secretion of milk, which changed almost immediately from coming drop by drop to a rapid stream.

(3) *The technique used by H. B. Fell is not very different from the usual techniques of cell culture. The nutritive medium is composed of a mixture of embryonic extract and blood plasma which ensures the coagulation of the medium and the organ is then placed on the surface of the coagulum so obtained. An extract of chicken embryo is prepared as follows: embryos taken from eggs which have been incubated for nine days are ground up and reduced to an exceedingly fine pulp. This pulp is diluted in a*

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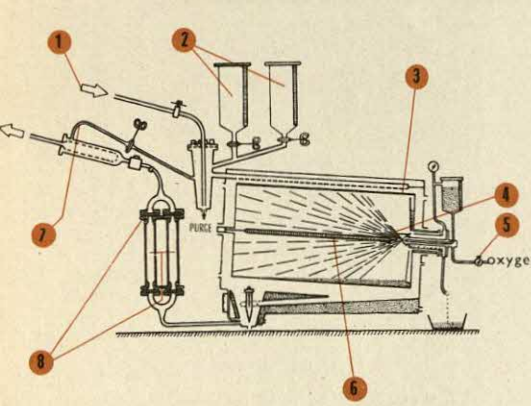

Fig. 5. Diagram of the heart-lung machine devised by J. A. Thomas, and used at the present time for intracardiac operations. 1: return of venous blood; 2: blood reservoirs; 3: revolving pulmonary membrane; 4: injection of a mixture of buffered saline and oxygen; 5: oxygen; 6: infra-red heating element; 7: output of arterial blood; 8: filter-pump of the artificial heart (photo J. A. Thomas).

stimulated, may even be smothered by the rapid proliferation of the former. By contrast, a medium that allows only a moderate amount of cell-division to take place, keeps the complex structures of the organ in their proper cohesion and relationship. This difference in the conditions of nutrition is a fundamental difference between cell and organ culture.

The culture of embryonic organs *in vitro* enables the following questions to be answered. (1) Can an organ develop outside the body? (2) How do two organs cultured together affect each other?

Culture of organs outside the body

Development consists of two processes: growth and differentiation.

(a) *development of the tibia*

A rudimentary leg bone, the tibia, may be taken from a young chicken embryo six days after the beginning of incubation. At this stage it is a rough model of the future bone, a short rod of uniform thickness in which cartilage is beginning to form in the centre.

This rudiment is placed in a suitable culture medium and the young tibia is seen to elongate as early as the first twenty-four hours and by the end of seven days it has doubled in length. Since it is anchored in the solid nutrient jelly by its enlarged ends, the ephyphyses, the bone often cannot grow straight and owing to its cartilaginous nature bends as it develops in the medium. At the same time it grows thicker and changes entirely into cartilage (fig. 7).

But the most remarkable fact is that the young tibia has acquired its characteristic morphology. Both ends have enlarged considerably and acquired projections and grooves which would normally enable them to articulate with the adjacent bones in the normal birds; one end with the femur, the other with the tarsus. If the femur and tarsus are cultured separately, they too develop articulating surfaces corresponding to those of the tibia. Thus, these bones look as if they had been modelled on each other; they have developed quite independently and yet fit together. In fact, it is an almost universal law of development that, after a certain stage, organs differentiate spontaneously, apparently without coming under any external influence, until they are ready to work together.

(b) *development of the genital glands*

Two rudimentary sex glands may be taken from two different embryos which are at the same developmental stage as that when the tibia was removed; both look like two narrow bands which are not distinguishable in any way, either by their external appearance or by their internal structure as seen under the microscope. They are placed in two culture vessels and if the two embryos happen to be of opposite sex, which cannot be told in advance, the two organs will be seen to develop quite differently according to whether they are male or female. If it is male, the gonad grows mainly in thickness, becoming cylindrical. If it is female, it spreads out on to the surface of the culture medium, increases in size, and is surrounded by a thin transparent fringe. These external differences are the same as those that the glands would have developed inside the embryo and correspond to characteristic structural differences between the testis and the ovary.

At first the reproductive glands of the two sexes have the same structure and may be described as bi-potential although, in reality, they consist of two rudiments: a peripheral or cortical region which is potential ovary, and a central or medullary region which is potential testis. However, by culturing them we can show that they contain factors which stimulate one of these rudiments and cause the other to degenerate. Thus the ovary develops as a result of the differentiation of the cortical part and the atrophy of the medulla, while the development of the testis involves the differentiation of the medulla and the atrophy of the cortex. Here, again, this is an example of differentiation without the action of external agents (self-differentiation), for the gland, even when left to itself, becomes sexually differentiated from an originally undifferentiated stage.

Combinations of organs

Organ culture makes it possible to bring about a great variety of unorthodox situations by putting organs together in different combinations. These organs may come from the same individual and normally are widely separated but suspected of being correlated in some way; or they may come from individuals of the same species but differing in age or sex, or even from different species or groups. In this way tissues that are normally separated either in space or time and would never under ordinary circumstances come into contact are artificially brought together.

Certainly, grafting can cause the tissues of different organisms to be united, but it is well known that the adults of higher vertebrates show an almost universal intolerance of grafts from another individual and the only grafts that are likely to be successful are those between tissues of the same individual (autografts)[5] or between identical twins. In contrast, embryonic organs from the most diverse sources are found to be remarkably tolerant of each other.

(a) *combining genital glands of opposite sex*

The genital glands of a chicken embryo which are removed before they are sexually differentiated normally develop into either ovaries or testes according to their sex. However, their fate is not irrevocably fixed at this stage and they can still react to external factors.

If we take the undifferentiated genital glands from the left side of two embryos and place them alongside each other in the same culture vessel, there is a 50 per cent chance that they will be of different sex, although this can only be verified by culturing their opposite numbers, the gonads from the right sides in separate vessels. Under these conditions the male gonad no longer develops according to its original sex, but comes under the control of the adjacent ovary, and, instead of becoming a testis, differentiates into another ovary, or at least into a gonad of intermediate sex (an ovo-testis).[6] Externally, this organ looks like an ovary and its internal structure is that of an ovarian cortex.

These findings indicate that while an embryonic ovary is developing it secretes a hormone which is capable of feminising a male gland. Moreover, a rudimentary testis can be changed into an ovary by culturing a male gonad on a medium containing several drops of a solution of female sex hormone. This shows how the methods of organ-culture may be used to test the action of chemical substances on particular organs.

(b) *combining tissues from different species*

One of the strangest combinations that can be attempted is to join the tissues of a chicken to those of a mouse. Such a union would be quite impossible to obtain by grafting between adult tissues, since the foreign tissue would be destroyed or rejected shortly after being introduced into the host.

However, there is no such incompatibility between embryonic organs. For example, if pieces of rudimentary testes from two different species are cultured on a nutritive medium, not only do they fuse intimately but their tissues even interpenetrate and become intricately intermingled. The connective tissue cells of the mouse, as though attracted by the chicken tissues, migrate far into the foreign organ and their paths can easily be followed on account of the characteristic microscopic appearance of the mouse cells. The epithelial cells of the two species often come close together and form mixed cords of mutual seminiferous tubules, in which both types of cell conform to the same structural laws. Thus true 'chimeras' are obtained, organs made up both of mouse and chicken tissues (fig. 6).

However, this co-operation only lasts as long as the tissue can survive *in vitro*, that is some ten to fifteen days, although this period can be prolonged to more than a month by grafting the mixed organ first into one embryo and then into another. Admittedly, such a composite organ never becomes adult or functional, but it is very remarkable to find that the cells of two species so different as a mouse and a bird should show such strong affinities when brought together. It is as if they were able to recognise each other as being the same type of tissue and constituents of the same organ, the testis.

Chimeras have been produced between other tissues, such as the lungs and bronchi of a chicken and a mouse, and also occasionally between different organs of the same, or even different, species. One may well wonder whether experiments, which at an early stage accustom the tissues of one species to live for a long time among cells of a different species, might not let tissues so acclimatised be grafted to full-grown animals of the second species. Such experiments have not yet been made.

(c) *combining chicken organs with mammalian cancers*

Leaving these chimeras, the next question is whether embryonic organs might not combine with cancerous tissues of adults of the same, or even different, species; for cancer cells have properties like those of embryonic cells, notably their enormous capacity for proliferation. In fact, cancer tissues of chicken, mouse and even human tumour cells are readily cultured on embryonic chicken organs. Cancer cells are the only adult cells that increase actively under such conditions, although it is true that the combination is not as harmonious as that between healthy organs, for the cancer cells are nourished at the expense of the others. A particularly favourable medium is provided by the embryonic kidney which is gradually invaded by the tumour which behaves exactly like a parasite.

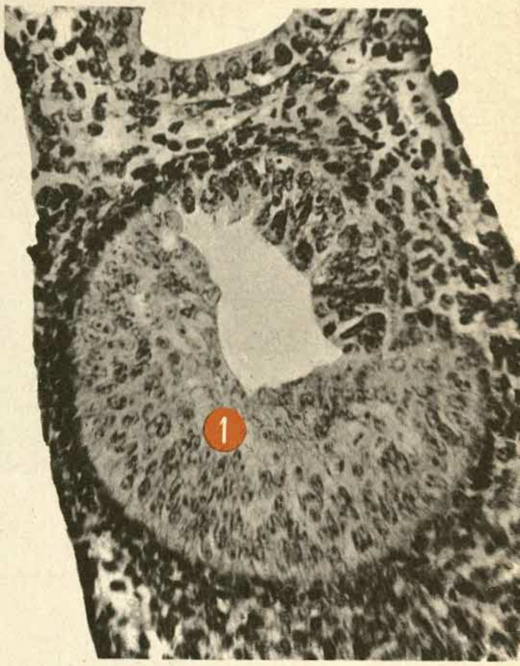

Fig. 6. Organ-chimera. Histological section of an association between the embryonic lungs of a chicken and a mouse. The bronchus (1) is formed of light chicken-cells and dark mouse-cells (mag. approx. × 400) (photo Etienne Wolff).

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solution of normal saline and the mixture centrifuged at 12,000 revolutions per minute for a quarter of an hour. The clear liquid above the debris represents the embryo extract. All these operations are undertaken with the most rigorous asepsis. Blood plasma generally comes from the blood of a horse or a cow: the fresh blood is removed aseptically and centrifuged in the presence of an anti-coagulant which causes the corpuscles to be deposited at the bottom of the tube, whilst the remaining fluid portion is the plasma. Serum differs from plasma in that it still contains the principle coagulant or fibrinogen.

(4) Agar-jelly. *Agar-agar is obtained from a seaweed. It lacks nutritive value, but dissolves in boiling water, and on cooling the solution sets into a jelly of about the consistency of apple-jelly.*

(5) An autograft *is a transplant of tissues in one and the same individual.*

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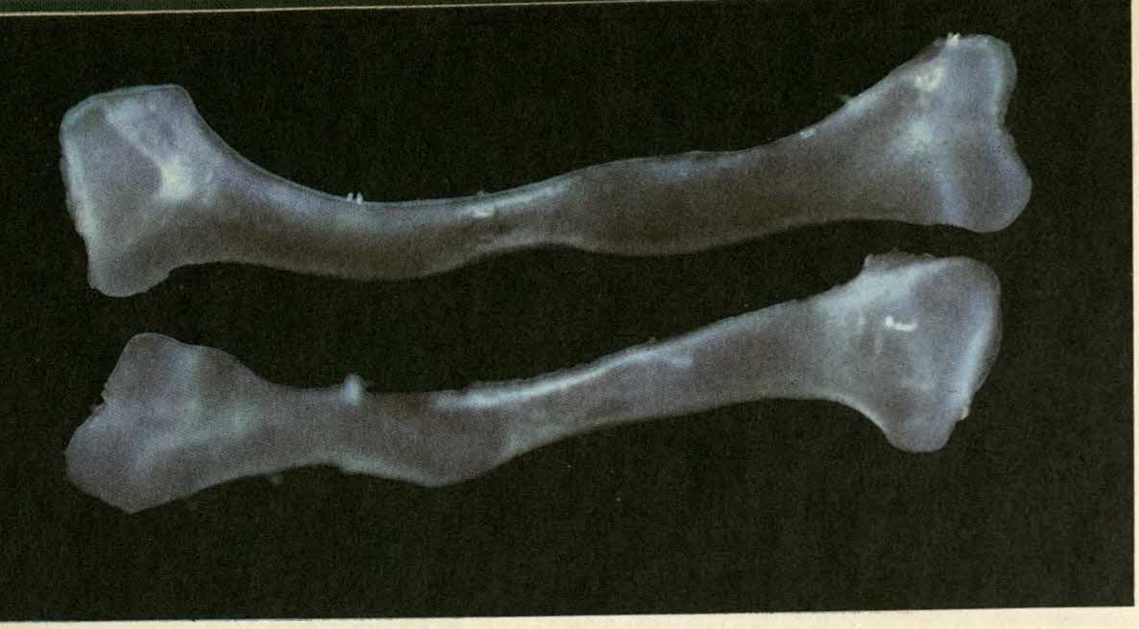

Fig. 7. Tibia of a chicken embryo aged six and a half days cultivated for seven days on the standard medium. It has grown from 3·3 to 6 mm, i.e., by 82 per cent; and gained 88 per cent in weight (mag. × 20) (photo Lod).

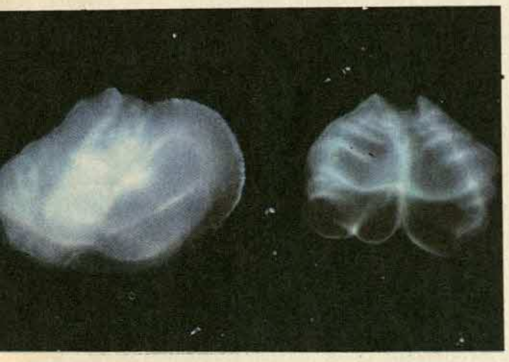

Fig. 8. Syrinx of a duck embryo. It is situated at the junction of the trachea and the bronchi. On the left, a male syrinx; it is markedly asymmetrical. On the right, a symmetrical female syrinx. Both organs have been cultivated for fourteen days in a nutrient medium (photo Lod).

It is interesting to find that cancer cells have such an affinity for the tissues of an entirely different species. This fact enables the process of tumour invasion to be studied under far simpler conditions than are ever found in the intact animal, for there are no nervous, circulatory or body fluids linking different parts of the organism and there is no possibility of metastases[7] or secondary growths being spread by the circulation. The cells of the organ and of the cancer can react with each other directly. Furthermore, the striking differences between the two types of cells enable the cancerous components to be identified unequivocally and the course of their migrations followed. Finally, it may be deduced from the results of these experiments that embryonic tissues, particularly those of the kidney, contain substances which are particularly favourable to cancer growth.

One advance towards our understanding of the factors governing development was made when it was found possible to substitute solutions of accurately known chemical substances for complex natural media, such as embryonic extracts and blood serum. The most important of these nutritive compounds are amino-acids (which are the basic materials for the synthesis of proteins) and sugars, to which various vitamins or growth substances may be added. Without going into complex experimental details, we can give a brief outline of how the development of organs varies according to whether they are given rich or poor nourishment, either in quantity or quality, and how it is thus possible to control development by altering the content of certain essential amino-acids, such as cysteine, arginine and histidine in the culture medium.

The vocal organ of birds, the syrinx, is a useful object for such investigations. It is situated at the point where the trachea divides into the bronchi and its walls develop a framework of cartilaginous arches which have a characteristic shape and number. In some species, such as the duck, there is considerable difference between the sexes (sexual dimorphism). In the male the syrinx is greatly enlarged into an asymmetrical box, while in the female it stays small and inconspicuous with distinct cartilages. The formation of these cartilages, like the appearance of sexual differences, is very sensitive to nutritional factors and it is remarkable to find that a rich source of nourishment favours the development of the male type while a poor one allows only the female type (fig. 8).

Conclusions

These researches have helped to determine the natural needs of embryos in general and the specific needs of their particular organs. A new field has been opened up in the study of the physiology of embryonic organs and the factors governing survival, growth and differentiation have now been recognised.

We have seen how culturing organs *in vitro* solves problems relating to the anatomy and physiology of the embryo, by simplifying natural conditions and reducing the number of factors that are involved in experiments on whole embryos. That is, the method eliminates the influence of the whole organism and its parts. But are we entitled to conclude as some people did in the enthusiasm aroused by the first news of these discoveries that an animal can be taken to pieces? Yes, provided we do not assume that it can be put together again; for an animal cannot be taken apart and reassembled like a machine. Its parts cannot be counted, nor can they be separated from each other without damaging their integrity and their connections.

We can speculate as to how research on grafting may benefit from the results of organ culture. For we know that grafts of embryonic tissue are better tolerated by adult animals than grafts of older tissues. Few organs have been kept alive for more than a few weeks, but it may some day be possible to culture them, or keep them alive after culture for several months or even years. This procedure has already been attempted with some success in animals and if applied to man could lead to the establishment of 'organ banks' of tissues available for grafting. But perhaps we are looking too far into the future.

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A homograft *is a graft between two individuals of the same species and the same race.*

A heterograft *is the grafting of tissues from one species (or race) to another.*

(6) *A bisexual or hermaphrodite gland. This name is given to a reproductive gland having both male and female features.*

(7) Metastasis: *Cancer cells can leave their original site and travel by the blood-stream to all parts of the body. They multiply in an uncontrolled way, like the primary growth, thus forming secondary tumours or metastases.*

From organ to organism

Pierre-Paul GRASSÉ

What by convention is called living matter is a heterogeneous mass with an infinitely complex structure. It is differentiated into parts which are responsible for carrying out the activities of life, either alone or in co-operation with one another and it is remarkable to find that the functional equipment of living things is the same throughout the plant and animal worlds. Thus, parts of the cell such as the mitochondria, Golgi apparatus, endoplasmic reticulum and chromosomes, have the same fundamental structure in all forms of life: they are immediately recognisable. Such differentiation and the heterogeneity it inevitably results in, is characteristic of life, at least as it occurs on this planet; for since its origin life has evolved towards greater complexity and differentiation associated with the development of specialised functional parts. The viruses are an exception, since they are not autonomous and thus cannot perform these activities for themselves.

The unicellular state and differentiation of parts

Single-celled organisms are included in a group called the Protista which may have either plant or animal characteristics. The Protophyta are plant-like in that they are capable of living on minerals alone, that is, they are fundamentally autotrophic. The Protozoa are animal-like in that they must be supplied with organic compounds which have already been synthesised by autotrophic organisms, that is they are heterotrophic. The simplest of the unicellular organisms are the amoebae which, although many of them look alike as a result of convergent evolution, belong to several classes of Protista and form neither a single unit of classification nor a natural line of evolution.

Like all living things, an amoeba has the following characteristics: it feeds, that is, it assimilates the nutrients it needs for its own synthesis; it excretes, that is, it gets rid of the waste products of its metabolism; it reproduces, either sexually or asexually; it moves; and it is sensitive (some biologists say irritable) that is, it can react to certain stimuli. In an amoeba this latter property is often associated with quite complicated behaviour.

Apart from its cell components an amoeba shows little structural differentiation. It can form pseudopodia, temporary projections of protoplasm which can be pushed out and then withdrawn and these aid the creature to move about from one place to another or to absorb its food. It has a contractile vacuole which, by filling up at intervals and emptying to the outside, rejects excretory products and maintains the constancy of the internal osmotic pressure. Thus the amoeba thrives on a minimum of specialisation. However, although the amoeboid way of moving and feeding is not a universal cell characteristic, it is fairly widespread among living things.

Many types of Protozoa have undergone a very marked differentiation of their parts and developed minute organs, called organelles. For instance, the Flagellates which are parasitic, symbiotic, and flagellated; and the Infusoria, which are ciliated, have many organelles: those for movement (flagella, cilia and contractile fibres); for support (skeletal plates and axostyles); for secretion (trichites); for sensitivity (tactile rostrum and sensillae); and for excretion (contractile vacuoles).

However, in spite of such internal differentiation, unicellular organisms have not passed beyond a fairly low level of organisation. The causes of this evolutionary stagnation are not clear, but are probably different from those which have been suggested up to now. To speak of evolution towards greater differentiation is to give a mystical interpretation of the facts which, without explaining them, appeals to some Aristotelian entelechy or Bergsonian élan vital.

It might be thought that the unicellular state must necessarily limit the size of an organism and condemn it to remain a primitive structure, but this is only partly true; for there are large single-celled green algae,

(1) Synergy: *Working together of various organs and muscles. The interaction of several organs to accomplish a single function.*

(2) Chondriome, Golgi apparatus, ergastoplasm, chromosomes: *see p. 59, 'From cell to tissues'.*

(3) Autotrophic, heterotrophic: *See p. 68, 'Culture of cells in synthetic environments'.*

(4) Habitus: *external aspect, peculiarities relating to the behaviour of particular bodies.*

(5) Convergence: *Convergence is the resemblance either simply in external appearance, e.g.: the general shape of the body and the presence of a triangular dorsal fin in sharks (Selachii), the ichthyosaur (extinct reptile) and the dolphin (Cetacea), or in organic structure, e.g.: convergent adaptation in the frog, the crocodile and the hippopotamus, with nostrils and eyes just showing above the level of the water when they swim, occurring in living beings which do not belong to the same class.*

(6) Stimulus: *everything which arouses a reaction or excitation in the organism.*

(7) Pseudopodia: *Retractile and very mobile extensions of the protoplasm which change form and are used in locomotion or feeding in cells such as the leucocytes or certain protista like the amoeba.*

(8) Vacuoles: *fatty or aqueous inclusions in the living cell containing several substances, either in solution or precipitated. The vacuole is said to be 'pulsating' when it swells and empties alternately, thus ensuring the constancy of the internal surroundings, in particular that of the osmotic pressure which is proportional to the concentration of dissolved substances in the protoplasm and inversely proportional to molecular weight.*

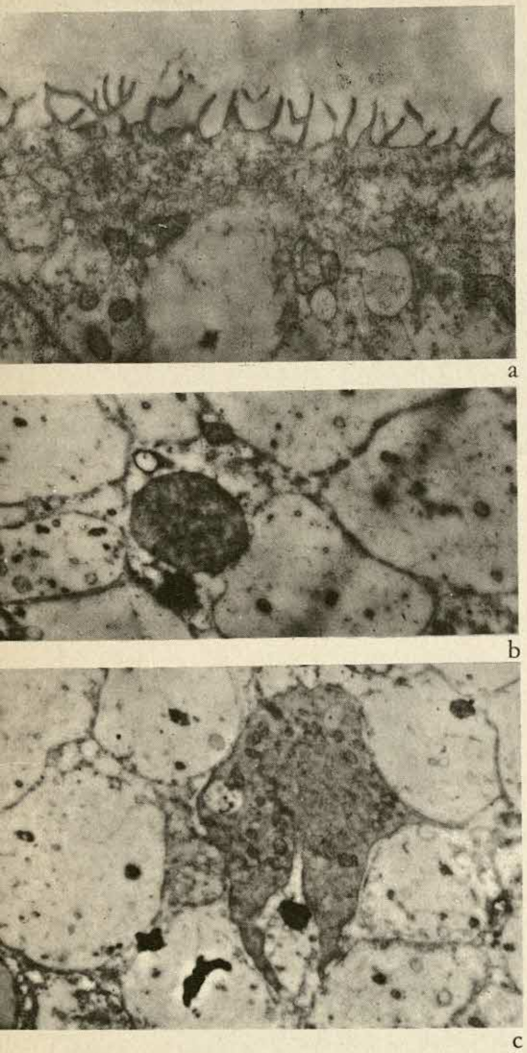

Fig. 1. Section of a Myxosporidian, Sphaeromyxa sabrazesi, *which lives in the gall-bladder of the sea-horse although classified as a protozoon, this creature has two kinds of cells, the perishable somatic ones and the potentially immortal germinal cells. (a) periphery of the plasmodium; (b) section through the somatic nucleus (vegetative); (c) section through a germ cell (a: mag. × 21,750; b and c: mag. × 15,625) (photo Pierre P. Grassé).*

(9) Amoeboid movement: *the ability of the cell to move and feed itself like an amoeba, that is, by putting out pseudopodia.*
(10) Symbiosis and parasitism: *in symbiosis the partnership is one of two or more organisms which live and develop together by mutual support. In parasitism, one partner lives at the expense of the other.*

Caulerpa and *Acetabularia*, which have rhizoids, a stalk and fronds, so that by convergent evolution they look remarkably like higher plants. Other unicellular organisms which reach a considerable size are the Mycetozoa and Foraminifera groups of Protozoa. However, these are multinucleate: each nucleus controls a portion of the surrounding cytoplasm and in all such protozoa, the nuclei have strictly equal functions.

Smallness of size does not prevent organ formation for the larval forms of many animals, the Rotifera, the Gastrotricha, and the Microhymenoptera are often smaller than an Infusorian and even than an amoeba, yet they have a complex organisation and are equipped with the same systems as larger animals.

Several protozoa have shown some tendency to differentiate, but from the evolutionary point of view this has come to a standstill. Such is the situation with the Cnidosporia. These protozoa are neither composed of a single cell, nor of a group of equivalent nuclei, but contain clearly characterised cells within a mass of equipotential nuclei (a *syncytial* mass). Each cell is surrounded by its own membrane and their cellular nature has been confirmed by the electron microscope. They are the reproductive cells, or *gonocytes* (fig. 1). In addition, the spore which serves to disperse the organism is composed of three kinds of cells: *valve cells; polar capsules* constructed like the *cnidocysts* of Cnidaria; and a binucleate amoeboid *germ cell.*

Apparently this type of differentiation has not been the starting point for an evolutionary line because the different cells are not grouped into coherent systems.

The multicellular state and the development of organs

Certainly, the multicellular state is an essential prerequisite of a higher degree of differentiation, but in order to build up organs the separate components must be held together by special properties of their membranes. The grouping of similar cells, specialised for a particular function, can then lead to the formation of organs. In physiological terms an organ may be described as a system of cells specialised to carry out a particular function.

In a unicellular organism all the basic functions occur together and take place more or less alongside one another. Rather than new activities being developed in a multicellular organism, the different functions tend to be separated and subsequently specialised organs are evolved.

However, the separation is compensated for by systems of communication which serve to co-ordinate the activities peculiar to each organ or system of organs. There is, for example, the circulatory system which distributes nutrients to the whole animal by means of an internal fluid, as well as transporting the respiratory gases (oxygen and carbon dioxide), the secretions of the *endocrine glands*, and the nervous system.

While differentiating from their primitive condition animals have evolved from the unicellular to the multicellular state and in consequence each major function has been undertaken by a special organ.

Living activities may be grouped into three categories:

1. *Nutritional Activities*, which provide for the maintenance of the metabolism and growth of an animal and include the following systems: digestive, respiratory, circulatory, secretory, and the endocrine glands.
2. *Co-ordinating Activities*, which provide the basis for the relationship of the animal to the outside world, in other words, its behaviour. Two main groups may be distinguished: the motor activities which are undertaken by the muscles, bones, and joints; and the sensory activities carried out by the sense organs. The nervous system acts to co-ordinate the information received and directs the motor responses to stimuli.
3. *Reproductive Activities*, which are carried out by the gonads and their accessory organs.

Specialised organs

However, although organ formation cannot take place without cellular differentiation, the latter can take place without definite organs being produced. The sponges illustrate this point. These animals live on microscopic organisms filtered from the water passing through the bodies which are penetrated at intervals by canals and pores. This type of feeding is *microphagous*. The differentiated cells form a fairly irregular network, or stroma, and the only structures corresponding to an organ are the groups of oval cells peculiar to sponges, which each have a flagellum that beats inside a kind of hyaline protoplasmic collar and serves to capture the prey. The external covering or tegument of a sponge might also be likened to an organ (fig. 2).

Specialised organs make a tentative appearance among a few diploblastic (two-layered) animals such as some of the Cnidarians. Siphonophores possess a rudimentary respiratory system and certain medusae have the outlines of a central nervous system in the form of a ring of neurones round the base of their tentacles (fig. 3).

Some Cnidarians form colonies made up of individuals which are joined to each other in various ways, for example, by means of their hollow stems or stolons. Both free-living and fixed colonies are of interest in that instead of having organs specialised for a particular purpose, they have specialised individuals. Thus there are vegetative individuals (dactylozooids), feeding individuals (gasterozooids) and reproductive individuals (gonozooids).

There is a tendency to attribute the poor degree of differentiation into organs of the diploblastic animals to the fact that they are made up of only two layers. But although on the face of it this seems quite probable, it is contradicted by the fact that some triploblastic (three-layered) animals reproduce asexually by buds of only two layers.

Be that as it may, truly differentiated organs only appear among the triploblastic Metazoa (multicellular animals), so that the presence of the third layer, the mesoderm, plays a major role in organ development.

Cephalisation

The morphology and physiology of an animal is in fact determined by its relationship to the external world; for the sensitivity of both unicellular and multicellular free-living organisms is not equally distributed, but reaches a maximum in the anterior region which tends to develop into a head region, that is, to undergo *cephalisation.* Even among many Protozoa, such as the ciliated Infusoria and the Flagellates, the anterior end is more sensitive than the other parts of the body. This end corresponds to the head of the Metazoa or multicellular animals. According to the theory of gradients put forward by C. M. Child, those ciliated Infusoria and Flagellates which have a differentiated anterior end, have a gradient of sensitivity, with the front end having maximum sensitivity and the hind end minimum sensitivity. This has been shown to coincide with a gradient of metabolic activity; for L. H. Hyman's experiments have shown that the most active metabolic regions (which are at the head of the gradient) are the first to be attacked by toxic substances such as potassium permanganate and potassium cyanide. There are other gradients, in particular a dorso-ventral, that is one from back to front, which is very important with respect to both the anatomy and behaviour of the organism.

Cephalisation is seen clearly only among the triploblastic metazoa. The head differentiates to become the region which is richest in sensory receptors, performing functions such as touch, hearing, and taste, and it is also the site of the organ for taking in food, that is, the mouth. This is one of the most outstanding features of animal organisation: the fact that in all the free-living triploblastic metazoa, the anterior-posterior gradient of sensitivity reaches a maximum at the anterior end which becomes a head for carrying out both sensory and nutritional functions.

In order to understand better the nature of biological phenomena, one always tries to refer to simpler organisms where the fundamentals are not obscured by too many additional features. But do such organisms exist? It is unlikely. Species with differentiated organs and simple anatomy are uncommon among present day animals and usually the ones we know are forms which have degenerated because they have adopted a particular mode of life, rather than those which have preserved their primitive simplicity.

However, the larval stages of species which have a complex structure as adults, might be said to have a fairly primitive organisation. This is illustrated by the trochophore larva of Annelids and allied groups (Sipunculids, Echiurids and Molluscs), and the nauplius larva of the Crustaceans. But this concept can be misleading, for the larvae cannot be called archetypes: they are usually transitory forms adapted either to a pelagic or a simple aquatic life and to the dispersal of the species and they are the result of an extremely long evolution.

A trochophore larva is shaped like a top with a large anterior end about 1 mm across (fig. 4). It bears a tuft of long flagella beneath which lies a nerve centre called the syncipital organ and below this several nerve rings are arranged in tiers close to the ectoderm. These rings are united to the syncipital organ and to one another by longitudinal nerve fibres. Still further inside the animal there are muscle fibres which pass from one pole to the other. The gut is bent at right angles with a mouth at the side and a terminal anus; the whole structure consists of various cell types. Excretion is carried out by the complete renal organs or protonephridia, which are tubes closed at their internal end by a cluster of flagellated cells in the lumen of the tube; the external end consists of an open pore. The posterior region of the digestive tube

(11) Polyenergid: *an energid is made up of a nucleus and cytoplasm placed physiologically under its dependence. An organised being having more than one energid is called a polyenergid. Energids are said to be equipotential when they all have the same properties and can all fulfil the same role.*

(12) *See Note 14 below.*

(13) Stroma: *the denser part of a living tissue.*

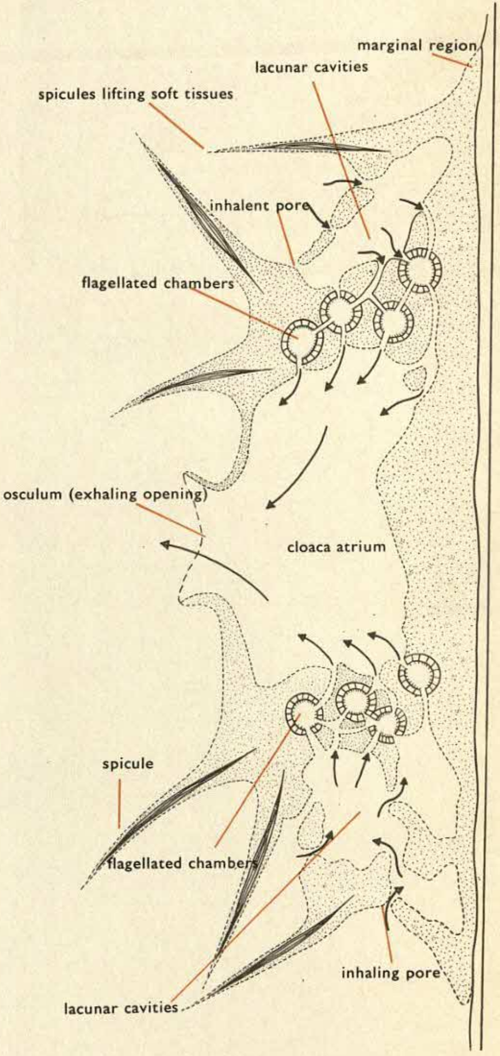

Fig. 2. Section of a Sponge, an animal which filters water.

(14) Choanocytes: *special cells found mainly in sponges. Of oval shape, they have a flagellum which beats inside a hyaline protoplasmic collar, a device which serves to capture the microscopic prey on which the sponge feeds.*

Fig. 3. The jelly-fish already shows an elementary nervous system (Atlas-Photo).

is flanked by two large cells which, having a potent capacity for division, give rise to the third layer or mesoderm in the form of two symmetrical lateral cell masses. The space between the body wall and the digestive tube is filled by a fluid in which some free cells are present and acts as a circulatory system.

Here, both the antero-posterior gradient and a bilateral symmetry are clearly marked and the organism's functional unity is dependent on a nervous system which is of greater anatomical importance than the classical descriptions of it suggest.

The nauplius is the larva which arises from the egg of many Crustaceans, particularly those species classed as Entomostraca. It is a minute organism bearing three pairs of swimming appendages. It has a light-sensitive organ (photo-receptor) both at the front and on the back. The nervous system has two centres, one above and the other below the gut, both being united by two nerve cords which form a peri-intestinal collar (fig. 5). There are clearly differentiated muscles, digestive system, and excretory organs. Some of the cells from the masses at the rear end of the digestive tube will give rise to the segments of the future adult.

The unity of multicellular organisms

All metazoa are made up of a large and variable number of cells, each of which is an anatomical and physiological unit. A higher category of biological units is provided by the organs which are composed of specialised groups of cells. Thus an organism appears to be a structure of many apparently independent parts. In order to establish and maintain its own unity or individuality under such conditions some co-operation and co-ordination between the component parts is essential.

In reality, a living multicellular organism is no more an aggregate of cells than a bundle of organs. It is a system in which all the component parts are jointly responsible to one another, and the physiological and anatomical arrangement serves to enforce the co-ordination and co-operation of the parts.

The nervous system and individual unity

The nervous system, in particular, has many functions within an organism all of which are more or less directly responsible for the maintenance of the unity of the individual. These may be classified into four groups:

1. The nervous system establishes relationships between organs by receiving messages from the various organs by way of the afferent nerves and sending out messages by way of the efferent nerves. The functioning of a particular organ is adjusted to that of one or several other organs, and the nervous system acts as a regulator to harmonise their activities. Among the Coelomates, and perhaps even in several Acoelomates, there are special nerve centres which perform this role. According to the zoological group under consideration, these may be termed the stomogastric nervous system, the sympathetic nervous system, or autonomic nervous system and so on. These centres control the so-called vegetative activities of an organism, that is those which do not concern the behaviour resulting from the peripheral stimulation of the animal. Information to the sympathetic nervous system comes from sense organs which receive messages, mainly of a chemical nature, from the viscera (*interoceptors*).

2. On the other hand, another part of the nervous system links the peripheral receptors *(exteroceptors)* with the effector organs, the muscles and the glands. Thus the nervous system collects information from outside the animal by means of sense organs and sends out orders to effector organs so that they may respond to significant stimuli received.

3. The nervous system has another very important function for the maintenance of organic unity and the assertion of individuality in behaviour. This is its preservation of the records of the experiences the animal has undergone in what we know as memory. Memory constantly intervenes in animal behaviour, the simplest form being the conditioned reflex.

4. Not only can the nervous system influence other organs by sending out nerve impulses, but it also discharges substances, which it synthesizes, into the bloodstream or sends them directly from the axons of the nerves to the organs concerned. These substances, other than acetylcholine, have not yet been chemically identified but examples of their action are provided by the well-known neurosecretory role of the hypothalamus which stimulates the pituitary. In insects the *pars intercerebralis* of the brain produces a secretion carried along the nerves to the *corpus allatum*.

Thus besides the reception, integration and emission of nerve impulses, the nervous system has an additional glandular function in that it secretes like an endocrine gland.

(15) Diploblastica and triploblastica: *the diploblastica are animals in which all parts derive from two germinal layers: the ectoderm and the endoderm. In the triploblastica, the organs are derived from three germinal layers: the ectoderm, the mesoderm and the endoderm.*

(16) Apical-basal gradient: *Gradient which decreases from the front region (apex, head) to the rear (base, tail).*

(17) Coelomata: *animals having a general body cavity or coelom which arises in the mesodermal mass of the embryo: their external or parietal layer is the somatopleur, the internal or visceral layer is the splanchnopleur.*
Acoelomate: *without a true coelom.*

(18) Neuraxon or axon: *Extension of the nerve cell or neuron, in the shape of a loose cylindrical filament ending in fibrillae which link the neuron to one or more other neurons or to a motor or glandular organ for transmission of the nervous impulse.*

The endocrine glands and individual unity

The nervous system is not the only system which ensures the functional unity of the individual. Endocrine glands have been found in all Coelomates. These are organs that synthesize substances called hormones which they discharge into the body fluids contained either in a system of vessels or in intervisceral spaces. The substances produced are distributed to the whole organism dissolved in a fluid, which may be blood, lymph, or interstitial fluid. The chemical composition of most vertebrate hormones is known. They have a definite physiological and morphological effect on cells sensitive to them.

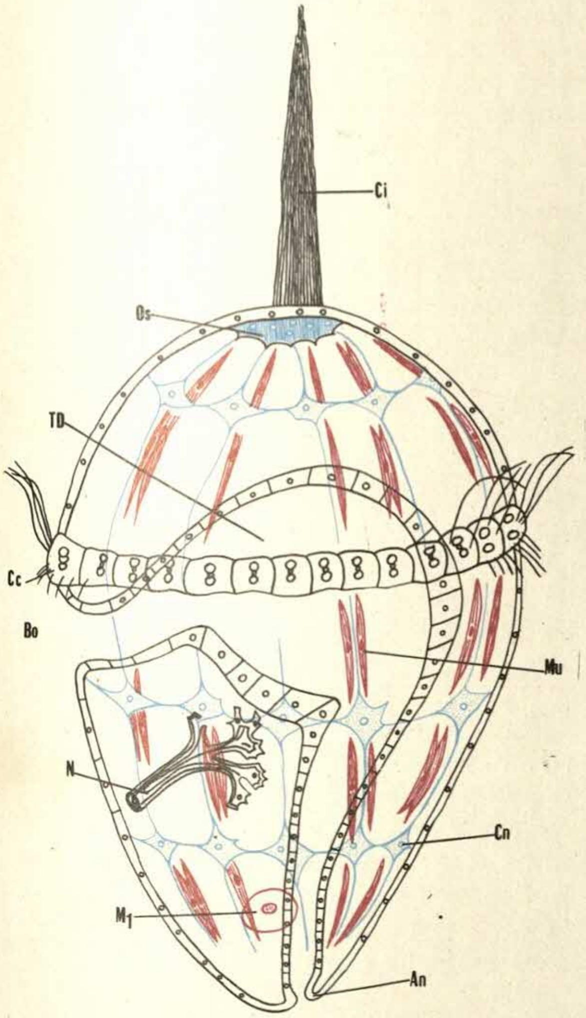

Fig. 4. The trochophore, or swimming larva of the annelids: An: anus; Bo: mouth; Cc: ciliated band; Ci: sensory cilia; Cn: superficial nerve cells; M_1*: left initial cell of the mesoderm; Mu: contractile cells; N: protonephridium; Os: apical organ; TD: digestive tube. Actual size: less than one mm.*

Hormones play a very large part in morphogenesis, that is, in the development of the various parts of the body. The inductive action brought about by some groups of cells during embryonic development has even been compared to that of hormones. This is a plausible interpretation; for embryonic hormones take part in the formation of the reproductive organs of vertebrates—for example, they are of the same nature as those produced by the ovary and testis of adults.

The early development of the individual depends on successive induced responses which take place in a strict sequence, a good example of organic integration.

The endocrine glands have an action on particular organs and their activities. Endocrine glands may appear to have a considerable degree of autonomy but, in fact, each is under the control of the nervous system and other endocrine glands. For example, the thyroid gland is dependent on the pituitary gland, which in turn is dependent on the hypothalamus at the base of the brain. It is also under direct nervous control. The thyroid is a powerful correlating and regulating factor: the hormone it produces, thyroxine, takes part in metabolic processes.

This sketch which has been deliberately simplified shows how closely correlated the interrelationships between organs really are.

In order to explain and interpret his observations and experiments, the biologist carries out constant analyses: he isolates parts and studies their structure and function. Life is based on certain fundamental activities and if the individual unity and life of an organism are to be maintained it is essential that these activities are co-ordinated.

A hierarchy of functions

In general the anatomy of a triploblast includes the following systems: nervous, digestive, circulatory, respiratory, excretory and genital organs, frequently accompanied by accessory organs. Some anatomists and a larger number of physiologists have envisaged a hierarchy of organs, but this is a point of view which takes into account neither the complexity nor the intricacy of the functions involved. For example, there is no doubt that excretion can take place only after a certain cycle of metabolic operations has been brought about, but a chronological relationship does not imply a functional hierarchy of organs.

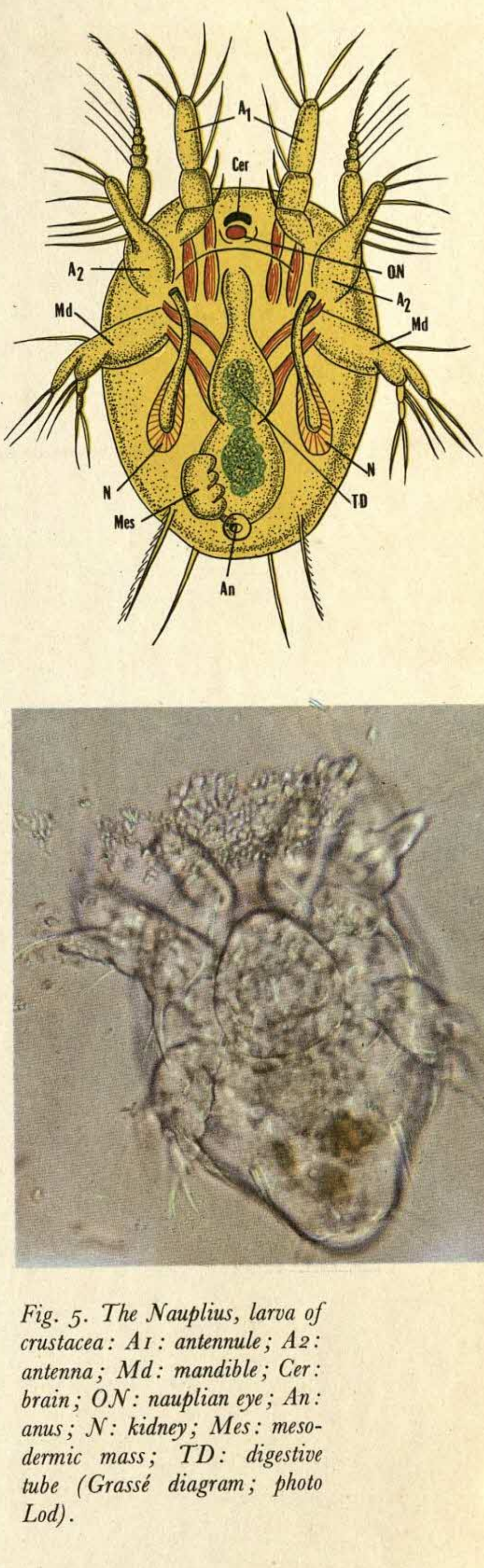

Fig. 5. The Nauplius, larva of crustacea: A_1*: antennule;* A_2*: antenna; Md: mandible; Cer: brain; ON: nauplian eye; An: anus; N: kidney; Mes: mesodermic mass; TD: digestive tube (Grassé diagram; photo Lod).*

(19) Hypothalamus: *part of the base of the brain situated beneath the optic thalamus into which the pituitary gland is inserted.*

On the whole, it would seem to be more objective to speak of mutual interdependence rather than a hierarchy.

The nervous system may be regarded as the master organ, but this is an anthropomorphic interpretation rather than a statement of the facts.

In principle, each function is regarded as corresponding to a particular organ. This is not quite true, for an organ often has many functions. For example, the lung takes part in respiration and lipogenesis; the liver carries out excretory, glycogenic (glucose producing), haematopoietic (blood making), detoxicating and other activities, while the pancreas is a composite organ, partly endocrine and partly secreting digestive hormones through a duct. Alternatively, the same functions may require several organs and this, in fact, is the usual situation in the case of reproduction, digestion, excretion and so forth.

Thus, as a rule, the different functions of an organism are neither separated not arranged in a hierarchy, but are rather interdependent and interlinked. These relationships reinforce in a remarkable way the functional unity of a living thing, its wholeness.

Regulating mechanisms

This 'symbiosis' or neuro-endocrine association provides the best example of the links between organs on which the physiological equilibrium is dependent. The system is thus the basis underlying regulation of the animal.

The following is an interesting example because it starts from a peripheral stimulation: if a female pigeon is kept in isolation it does not lay eggs, but will do so normally if it is kept either in the company of other male or female pigeons, or sees its image in a mirror.

We can interpret this by saying that the image of the pigeon is a 'significant' stimulus which, after being relayed through several nerve centres, excites the hypothalamus to secrete a substance which excites the anterior lobe of the pituitary gland. The pituitary then sends out an increased quantity of a gonadotrophic hormone which activates the ovary and initiates laying (fig. 6).

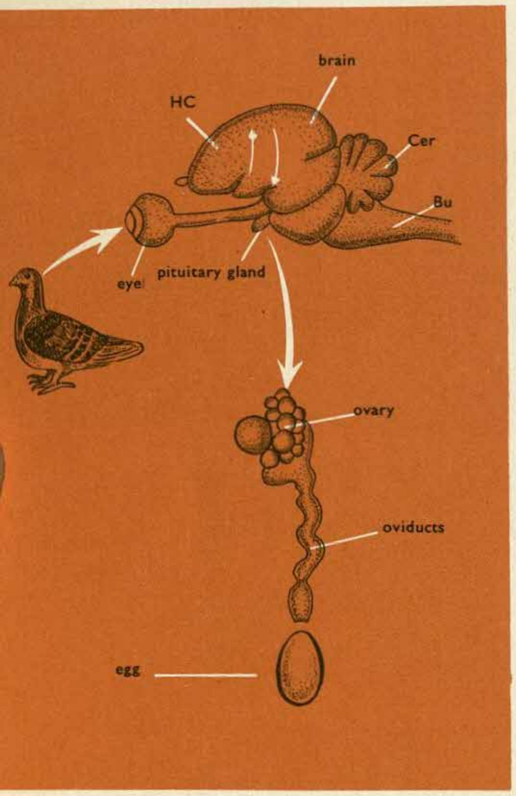

Fig. 6. Diagram showing how psycho-neurohormonal relations release the laying behaviour of a pigeon. Bu: medulla; Cer: cerebellum; Hc: cerebral hemisphere.

Another example is the respiratory centre which is sensitive to the carbon dioxide level in the blood and acts to control the respiratory rhythm. Physiology includes many such activities which work together and follow one another as a chain of reactions.

The maintenance of the physiological balance (homœostastis) is carried out by regulatory mechanisms, such as the glycogenic activity of the liver in controlling the glucose level of the blood. The regulatory mechanisms are very diverse and it is not possible to list them all here. Nevertheless, the one which takes place all the time by carrying information from the sensory receptors to the nerve centres should be mentioned. Without sensations and the appropriate responses which they initiate, an animal could not live nor be what it is.

Organs and the mode of life

If the animal world is viewed as a whole it is seen that the mode of life and behaviour of animals have profound repercussions on their anatomy and functioning. Species which are fixed permanently to a support, no matter what group they belong to, do not have a differentiated head region and tend to form colonies (Sponges, Hydroids, Madrepores, Bryozoa, Pterobranchs, Ascidia). They have few organs, although these may be present in their free-living larvae. Parasites have been either complicated (Infusoria and Flagellates) or simplified by loss of organs. The best examples of the latter are *Sacculina* among crustacea and *Taenia* (tape-worm) among the flat worms. The circulatory, respiratory, digestive and locomotor systems have entirely disappeared and, as if in compensation, the genital system is enormously hypertrophied. Very often the female or the hermaphrodite is no more than a sac producing and storing eggs. There is also an enormous development of absorbing surfaces when a parasite is bathed in a nutrient fluid, such as blood or chyle. For example, *Sacculina* has rhizoids, *Taenia* a much-folded integument.

Thus an organ depends on its function for its existence. When the activity is no longer required, the organ disappears. Besides present-day factors which affect the attainment of a particular form and the carrying out of certain functions, there are historic factors the importance of which should not be underestimated since all living things are the outcome of an evolution which has left its mark on their anatomy and physiology in thousands of ways. Time has played a more complicated part in biology than in the physical and chemical phenomena of inanimate nature, because of the highly heterogeneous structure of living matter and the fundamental role which time plays in the construction and organisation of its parts, as well as in preserving its specific heritage, by the mechanisms of heredity and the material continuity of its germ cells.

(20) *On the subject of 'hormones' see p. 143, 'Development of sexual organs in vertebrates'.*

(21) Lipogenesis: *formation of fat-producing bodies in animal or vegetable organisms at the expense of food.*

(22) Glycogenesis: *formation of glucose by hydrolysis of glycogen, held in reserve in the liver or muscles, as required by the organism.*

(23) Haematopoiesis: *the manufacture of blood cells.*

(24) Gonadostimulin: *hormone secreted by the pituitary which stimulates production of sex hormones by the ovaries and testes.*

(25) Sacculina: *a rhizocephalic crustacean parasite on crabs which, in the adult state, appears as a pouch hanging from the abdomen of the host.*

> The body dies little by little and part by part; its activity lessens by degrees, life fades in a series of slight changes, and death is only the final stage of this sequence, the last small change in life.
>
> BUFFON

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PART THREE

The possibilities and limitations of life

Buffon said that life begins to decline a long time before it is finally extinguished. In fact, as soon as the period of growth is over, most living beings start to age and slowly progress towards death. Their tissues become less active with age; their cells gradually cease to multiply and finally die. There are however some cells and some organisms which escape this fate and retain their original youth and vigour. Life can only continue on this earth because all living creatures are endowed either wholly or in part with this potential immortality. (Such immortality can only be achieved if the environment in which the creatures live remains favourable. Clearly life ceases and living matter returns to an inert state as soon as external conditions are sufficiently altered.)

Among the higher animals such as birds and mammals, the large majority of cells is doomed to deteriorate and die: these are the 'somatic cells', or soma. A small number of cells localised in the sex glands do not die and ensure the continuation of the species: these are the 'germ cells', or germ plasm. The soma, that is to say the whole of our bodies except the reproductive cells, may be considered as a temporary and perishable offshoot of the immortal germ cells. Why are the somatic cells unable to avoid ageing and death? Geriatrics has shown us how the vital functions progressively decline with age, but has not yet discovered the underlying reason for this decay.

In animals less highly developed than the warm-blooded vertebrates, there exist, besides the immortal germ cells, some somatic cells which are able to preserve their vitality and their capacity to divide and differentiate. These are the 'regenerating cells' capable of restoring a limb or a tail in the newt (Amphibian urodele) and any part of the body in planarian (turbellarian flatworm).

Certain unicellular organisms, such as paramecium, seem able to maintain their power of division indefinitely, and thus to live for ever. The freshwater hydra, which is growing all the time, can also be regarded as an immortal animal: certain of its cells are continuously multiplying, as others are progressively eliminated.

Finally, plants present an example of ageing which, unlike that of higher animals, is not irreversible. The cells of a plant always retain the ability to revert to the undeveloped state, and, if conditions demand it, their power of growth and differentiation.

All living beings, then, harbour this faculty for survival. Their potential immortality appears to be one of the fundamental characteristics of life on this earth.

Certain cells of the axolotl (left) or of the sea-urchin (right) are able to 'retain their youth'. That is to say, they are able to regenerate the organ of which they are part (photo Lod).

The possibilities and limitations of life

Françoise STÉPHAN-DUBOIS

The story of the Phoenix which rose from its ashes, wholly fabulous though it be, is not more marvellous than the discovery of which we are about to speak (regeneration). BERNARD DE FONTENELLE

Since the beginning of time, among all peoples, from the most primitive to the most civilised, prolongation of youth, rejuvenation and invulnerability—all attributes tending towards immortality—have formed a part of human ambition, as countless myths and works of art bear witness. Nevertheless, one form of artificial youth had not struck the human mind; regeneration,[1] which permits the replacing at will of any part of the body that has been destroyed or worn out. This phenomenon, described as 'miraculous' in the eighteenth century, and as worthy of science fiction by the modern press, remained almost completely unknown until the era of experimental research. Only the myth of the Hydra of Lerna touches on the possibility of regeneration; this fabulous multi-headed Greek serpent was able to grow two new heads for each head that was cut off. Hercules killed the Hydra by cutting off all its heads at one blow. However, this legend does not establish knowledge of regeneration, as it symbolised the struggle against the unhealthy swamp of Lerna, which had to be drained and set on fire repeatedly.

This ignorance on the subject of regeneration explains the enormous stir caused by the experiments of Trembley in 1740–1744, which revealed the extraordinary powers of regeneration of freshwater hydra,[2] which Trembley called 'organised bodies' because he was not sure whether to class them with plants or with 'insects'.[3] It is difficult to imagine how, in those far-off days, a biological discovery could arouse passionate interest amongst the educated public. This particular discovery mobilised the learned societies and provoked the spirited mockery of such critics as Voltaire.

In our time, on the other hand, the same public which is familiar with evolution, chromosomes and hormones, is almost completely ignorant about regeneration. Is regeneration a rare curiosity, a sort of monster? To observe regeneration, does one need expensive laboratory equipment, available only to the privileged few?

A worm regenerates its head

Not at all. Anyone can bring about regeneration and the best subject for demonstration is a little flatworm called *Planaria* (fig. 2), which is readily fished up from ponds and streams. Regeneration will occur in planaria even after the crudest operation performed by children, using a pencil, a small piece of wood as an operating table, and the sharpened blade of a penknife as a lancet. The cut worms are placed in a glass with some of their native water. They need no food or attention, just to be left in clean water in the shade.

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(1) *Regeneration is the reconstitution by an organism of a part that has been destroyed. Physiological regeneration is the normal replacement of cells, tissues or organs, which takes place continuously throughout life in all living beings, e.g., cells of the skin, hair, feathers. The mechanisms of physiological regeneration often mingle with those of normal growth. Accidental or Traumatic Regeneration is the restoration of a part of an organism after amputation. This phenomenon brings into play complex factors, which are gradually being revealed and explained by experimental research. The power of accidental regeneration varies appreciably with the species of animal. It is this kind of regeneration only which will be considered in this chapter. In the case of total regeneration a half or even a fragment of an individual reproduces the complete animal (a worm can regenerate its head). In regenerating an organ, the part lost is replaced by a similar part (a newt can regenerate a limb).*

(2) *See the chapter by P. Brien (p. 107), "Freshwater hydra"*

(3) *In the eighteenth century the term 'insects' was applied to numerous invertebrates.*

(4) *Planarians are small flatworms, widely distributed in fresh water; most of them have great powers of regeneration.*

In the laboratory, a fine paintbrush replaces the pencil, and a slab of paraffin wax serves as the operating table; accurate sections are cut by means of a fine scalpel or, better still, a lance-shaped needle made to the experimenter's specifications; shallow crystallising dishes are more practical than a glass. For a particularly careful experiment the worms are anaesthetised.

The regenerative capacity of planaria

There are several species of planaria among the flatworms; their colour varies from dark brown to white, and their length from a few millimetres to three centimetres. Planaria lie hidden under stones or dead leaves in most ponds or unpolluted streams.

Let us take for our example *Euplanaria lugubris,* so named because of its sinister aspect. It is shaped all in one piece, without divisions or constrictions. Its head is the dominant part, bearing the sense organs, the brain, two eyes and two olfactory grooves; but it does not include the mouth, which lies in the middle of the ventral surface. From the mouth a tube called the pharynx[5] can be extruded. The internal organs are embedded in a cellular mass called the parenchyma.[6] An organised circulatory system does not exist and the body fluids flow through the parenchyma cells (fig. 2).

If one sections *Euplanaria lugubris* a little behind the head (fig. 1A) the decapitated piece moves only very slowly and practically stops feeding. However, the wound contracts and heals up within a few hours, becoming surrounded by a white border. This blastema of regeneration[7] develops two small eyes five to eight days after the amputation. A few more days, and the brown pigmentation appears. Three weeks after the operation one cannot distinguish between the regenerated piece[8] and the original portion.

Euplanaria lugubris has been cut in all possible ways: in squares (fig. 1B), in rectangles (fig. 1C), obliquely (fig. 1D) and longitudinally (fig. 1E). A general rule emerges from the results: each fragment reconstitutes the lost portions in such a way as to progress most rapidly towards a normal animal.

Why can a planarian regenerate so perfectly? *How does it know* that it lacks a head and *know* when the new head ought to stop growing? These are the same problems that confront all embryologists as they observe the harmonious development of eggs, and their methods of research are essentially the same: firstly, to study the basic material (the cells of which the embryo or the blastema is composed), then to analyse the mechanisms of development or regeneration. The study of regeneration has, however, two difficulties peculiar to it and due mainly to the presence of the old part, which is a necessary condition of regeneration: on the one hand, the old part screens the new areas from observation or direct experiment, and on the other, we cannot be sure whether the appearance and development of the blastema may not also be the cause of the phenomena observed. Is the young blastema self-sufficient like the embryo? Or do the old and the new parts work together in close collaboration?

These are some of the questions which have to be discussed in the light of our present knowledge of the problem.

The totipotent cells

One must remember that the body of *Euplanaria lugubris* is filled with parenchymatous tissue the cells of which form an irregular mass of supporting material in which other cells remain free or can easily free themselves. These cells have been called by a dozen different names since their discovery, but we will call them simply the 'cells of regeneration'. They are spherical, their cytoplasm is homogeneous, their nucleus is very large with a bulky nucleolus. Their general appearance resembles that of embryonic cells before these have become specialised (fig. 7 A and B).

After decapitation, the wound becomes covered by a small pellicle of epidermal origin. The cells of regeneration in the neighbourhood of the cut float towards it and pack together to form a 'regeneration bud'. The regenerative material is so abundant that the cells do not have to multiply actively. From the fourth day the bud starts to increase in length, forming a blastema in which the cells differentiate[9,10] by degrees (fig. 3). They infiltrate the scar of amputation and become skin cells; they arrange themselves into muscle fibrils; they assume the characteristic pigment of the eye, etc. (fig. 4).

How—certain biologists have understandably objected—does an adult planarian with a completely organised body, often so old that it would not survive the winter, manage to possess cells capable of recreating everything and starting again from scratch? That an epidermis can form a new skin, the intestine a new digestive tube, the nervous system a new

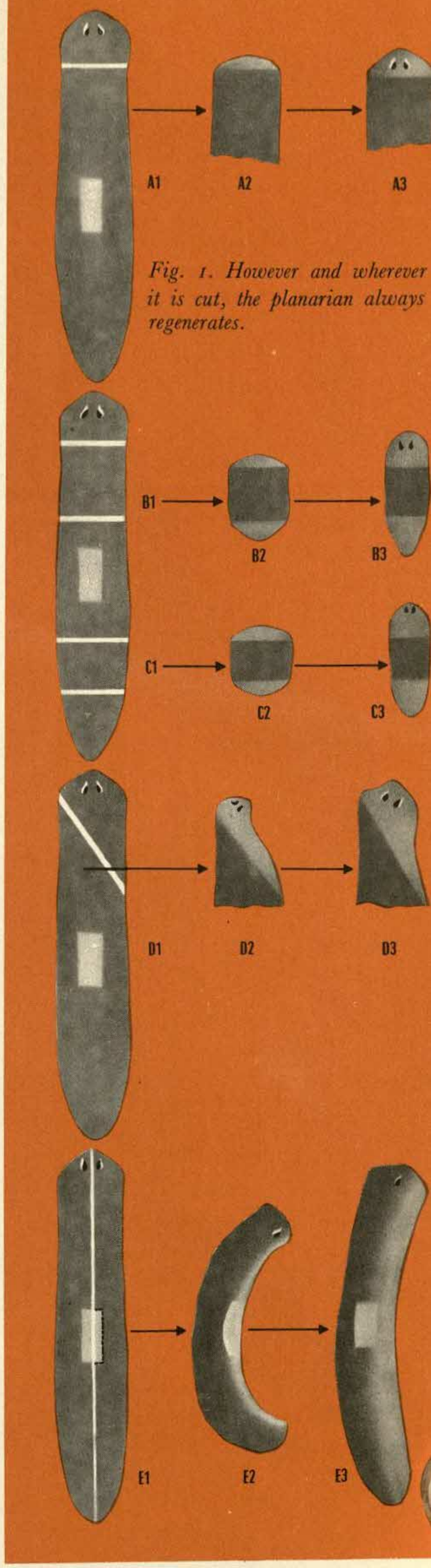

Fig. 1. However and wherever it is cut, the planarian always regenerates.

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(5) Pharynx. *The portion of the digestive tube leading from the mouth.*

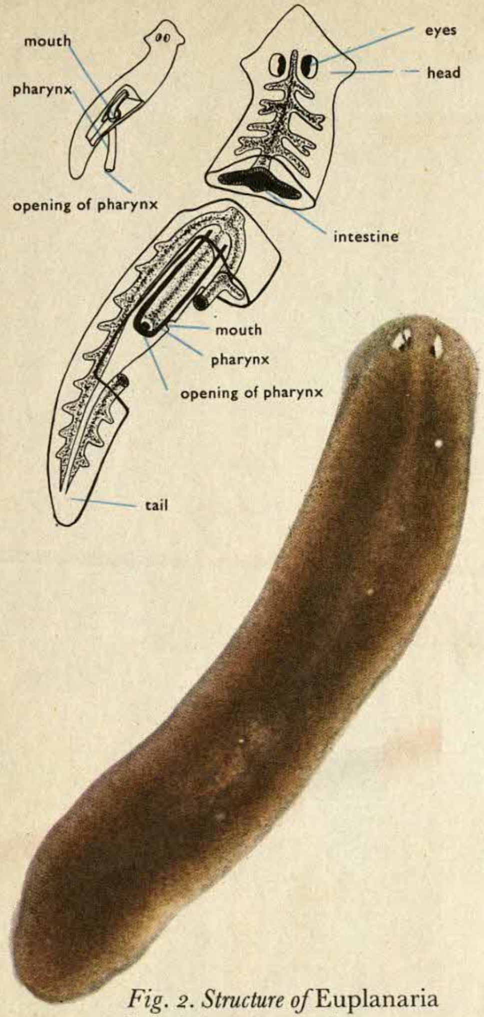


Fig. 2. Structure of Euplanaria lugubris *(photo Lod).*

brain, seems feasible, but to believe in the existence of '*totipotent cells*'[11] seems impossible.

It is certainly impossible to observe directly the movement of these cells and their transformation into cells of the blastoma. Thus it has to be shown by indirect means that the regeneration cells of *Euplanaria lugubris* are indeed endowed with this improbable faculty. The demonstration rests on the following principle. When a planarian is sectioned behind the head and then submitted to a suitable dose of x-rays, it remains alive for a certain time but is incapable of regenerating. The cells of regeneration are in fact more sensitive to x-rays than the other cells of the body; they die within a few days, whereas the differentiated cells survive for several weeks. There exists, then, a method for selectively eliminating the cells of regeneration.

If instead of irradiating the whole of the amputated part, only the part anterior to the pharynx is irradiated (fig. 5) or if the regenerative cells posterior to the pharynx are capable of migration, what is going to happen? At first nothing happens; the planarian does not regenerate and its skin gradually shows signs of radiation damage (blackish spots). Then after four weeks regeneration begins, and proceeds normally, so much so that the planarian regains all its vitality and the radiation lesions disappear. The planarian remains healthy and capable of further regeneration on future occasions.

The totipotence of these small cells has thus been proved. Nothing in front of the pharynx was capable of regeneration; everything must have come from the non-irradiated part posterior to the site of irradiation. It can be shown that healthy skin or intestine cannot move forward such a considerable distance; only the free cells of the parenchyma, which resemble each other in every way could filter between the maze of supporting tissue and congregate in the region of the wound. It takes a month for this migration to occur, which is not unusual on the biological scale.

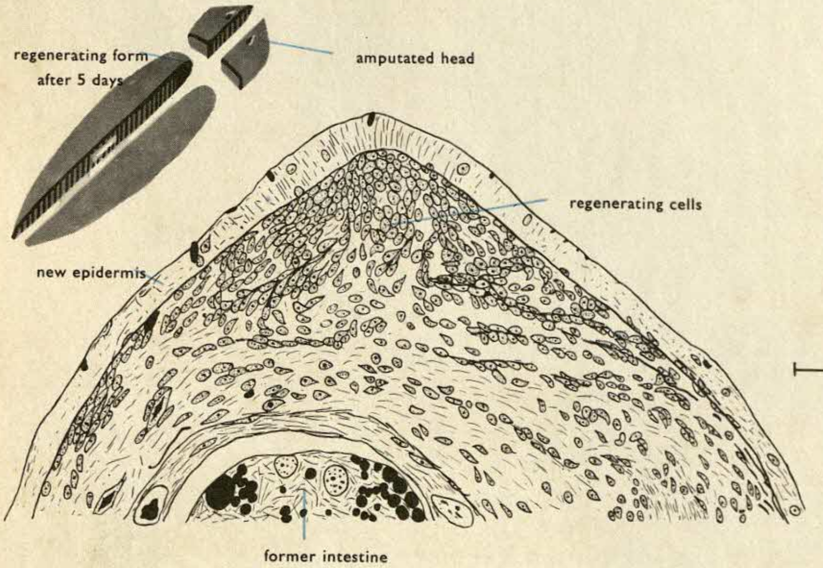
This demonstration proves the exceptional migrating power of the cells of regeneration as well as their aptitude for repairing existing old tissue. These cells retain eternal youth and form a valuable reserve within the body of *Euplanaria,* theoretically immortal like the germ cells.

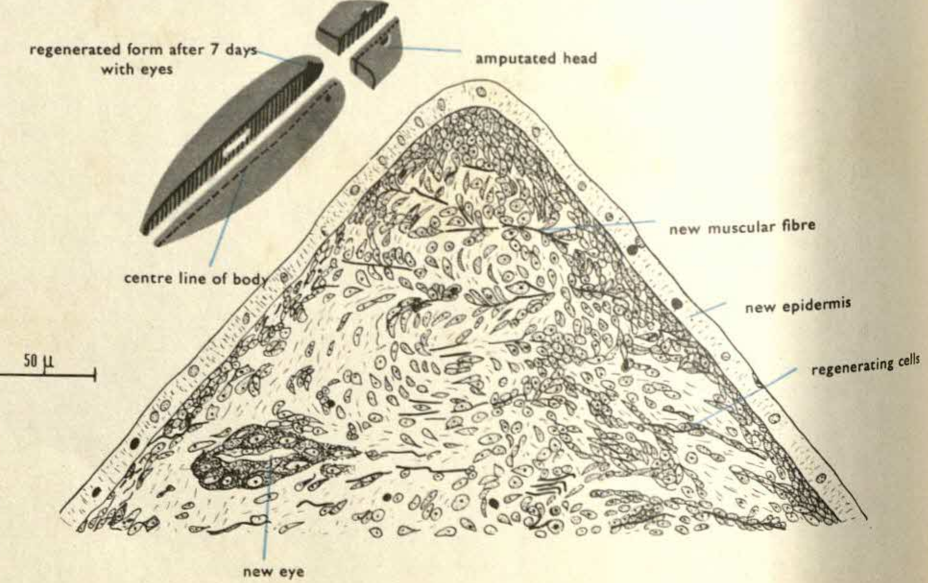
Some mechanisms of regeneration

Are these cells of regeneration also capable of organising themselves into a new part without guidance, in the same way as the egg develops into the embryo? Our answer must be qualified because regeneration is not just one reaction but a chain of complex reactions

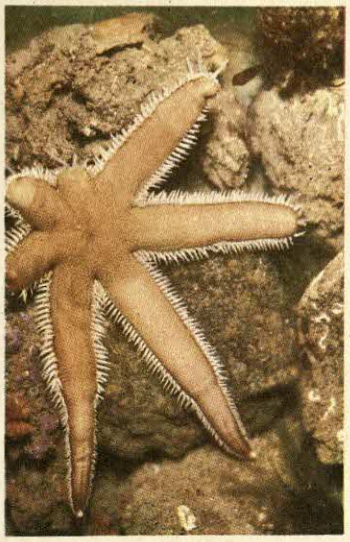
Fig. 3. Regenerating tissue: shown here, the regeneration of a head after five days; one can see elements surviving from the original portion.

Fig. 4. Regenerating piece after seven days; the formation of an eye can be seen. The epidermis has been completely reformed.





Stentor *(Mag × 140)*.



starfish

hydra

earthworm

newt

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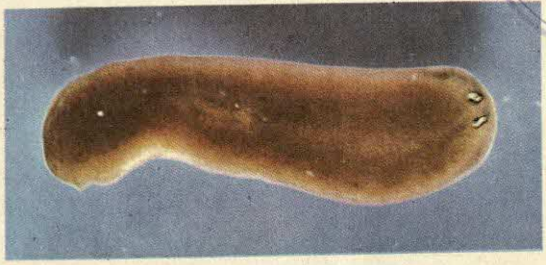


planarian

crayfish

snail

The old myth of prolonged or recaptured youth may remain an illusion for the higher animals like ourselves, but it is biologically true for the eight animals shown above, which have the power of regenerating the whole or part of their body. Stentor *(mag. × 140) starfish earthworm salamander planaria snail hydra crayfish.*

(Photos Lod)

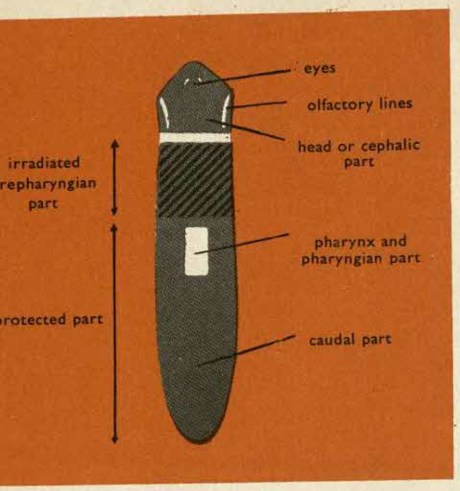


Fig. 5. Diagram of a planarian in which the region anterior to the pharynx has been irradiated.

(6) Parenchyma: *loosely packed tissue which supports the internal organs in certain invertebrates.*

(7) Blastema *(from the Greek 'blastemos': germ, shoot). A mass of cells with embryonic features. A blastema of regeneration is a bud filled with cells which organise themselves into regenerated tissues and organs.*

(8) Regenerate: *The creation of a part destined to replace a lost one.*

(9) Differentiation: *Transformation of a simple cell into a specialised cell. It is accompanied by the acquisition of visibly distinct characteristics. A differentiated cell is distinguished from other cells both by its appearance and its function. For example, certain free cells in the bone marrow of man differentiate into red corpuscles and white corpuscles. The former have a specific function in respiration, and the latter in the defence of the organism against infection.*

(10) Dedifferentiation: *The reversion of a specialised cell or structure to a simple type during regeneration.*

(11) Totipotence: *A cell is called totipotent if it is capable of developing into any type of specialised cell. The term applies equally to a small part which becomes detached from an organism and which can regenerate all the lost parts.*

most of which require the presence of the original portion of the animal. Some are completely independent. Two examples serve to illustrate the limitations of the cells of regeneration.

(a) *orientated migration*

The ability of cells to migrate is a simple phenomenon, but migration in a certain direction implies a co-ordination of movement. The regenerating cells ordinarily cannot be induced to move or orientate themselves in the direction implies a co-ordination of movement. communication is needed, some connection between the severed area and the migrating cells. Does the nervous system, that biological telephone, supply this? Or does some substance flow from the wounded tissues towards the healthy cells, carrying with it (by its chemical constitution or concentration) the message 'regeneration this way'? At present the latter hypothesis is favoured, but the possibility that the nervous system works in conjunction with the chemical substance, as in the neuro-secretory mechanisms of higher animals, has not been precluded (fig. 6).

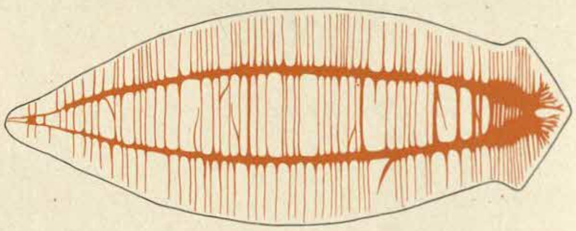


Fig. 6. The nervous system of the planarian.

(b) *the regeneration of eyes*

A small, widely distributed planarian, *Polycelis nigra*, possesses not two but several dozen eyes, arranged in a ring round the anterior pharyngeal region. This particular arrangement makes it possible to excise several eyes without touching the brain. The eyes regenerate within a few days. If one excises the brain at the same time as several eyes are excised (fig. 8) and prevents it from regenerating by repeatedly excising it again, then the eyes do not regenerate. The brain is necessary to the formation of eyes. It acts by secreting a chemical substance which induces[12] ocular regeneration. In fact, by grinding up the heads of these black planaria and centrifuging the liquid, one can extract a substance which will induce regeneration of eyes in the absence of the brain.

Generalising about cephalic regeneration from this phenomenon, one can say that the brain regenerates first; it then organises the regeneration of the eyes from cells which, left to themselves, would be incapable of forming an eye.

In orientated migration, there is some factor coming from the original part of the animal which initiates the movement of cells. In the case of the regeneration of eyes, the organising factor comes from the blastema itself.

Why does planaria regenerate so perfectly?

Dendrocoelum lacteum is a large species of flatworm attaining a length of three centimetres. If sectioned just behind the head, it regenerates very quickly. If cut midway between the eyes and the pharynx, it regenerates slowly and sometimes the head remains incomplete; behind this point the ability to regenerate diminishes abruptly. Regeneration of the head becomes impossible in the region of the base of the pharynx, although the tail regenerates immediately, regardless of the level of amputation.

As with *Euplanaria lugubris*, one can destroy all the cells of regeneration in the prepharyngeal region by the use of x-rays. If one decapitates the animal at the same time, a new head regenerates after a delay of several weeks, which corresponds to the length of time needed for the migration of healthy cells from the pharyngeal region. Thus cells which appear to be only capable of regenerating a tail can, if they are transplanted to a frontal region, regenerate a head.

This gradation of regenerative capacity shown by *Dendrocoelum lacteum* reveals a new fact: we can postulate the existence of a factor which enables the cells of regeneration to realise their potential capacity for regenerating a head. This factor only exists in the anterior region; or, what amounts to the same thing, there is a negative factor of some sort behind a certain level which inhibits[13] the regeneration of a head.

The following example demonstrates that such inhibitory factors really do exist. If one induces the formation of twin heads in *Euplanaria lugubris* and then cuts off one of the heads, in nine cases out of ten the amputation is not followed by regeneration. The remaining head inhibits the reconstitution of another.

These negative factors are useful to the planaria because without them regeneration would not take place smoothly; it would proceed in a disorganised fashion and produce monsters.

The head, with its brain, controls all the other parts of the body. In the regenerating animal, as soon as the head has become differentiated, the newly formed brain very probably produces a chemical substance which prevents the cells from producing another head. Each part of the regenerating animal and each part of the body remains under the influence of its environment, following a hierarchical order, each part dominating the part behind it.

To the question, 'Why does planaria regenerate so perfectly?' we can now reply that this extraordinary ability is due to the *simultaneous existence* of *cells of regeneration* capable of differentiating into any type of specialised cell, *relay factors* which ensure a liaison between the wound and the migrating cells, *inhibiting factors* which prevent disorder during regeneration, and, finally, *inducing factors* which ensure that organs regenerate in a predetermined order. It is not unreasonable to suppose that certain chemical substances may give rise to these various factors.

A newt regenerates a limb

Newts[14] are animals six to twenty centimetres long, depending on the species, and covered with a thin, moist, supple skin. The fore and hind limbs are very similar, except that the hands have four digits whereas the feet have five. Easy to find in the vicinity of ponds, they readily grow and breed in captivity and can go without food for a long time. Their eggs and larvae lend themselves to all kinds of experiments. Even in the adult stage they retain a capacity for regeneration exceptional among vertebrates. They can reconstitute eyes, nose, the tips of the jaws, limbs and tail. Possessing so many abilities, these little animals are much appreciated as objects of research in numerous biological laboratories.

Remove the forefoot of a newt, using aseptic precautions, and the wound heals in a few days. A regeneration bud appears at the end of the stump within two to eight weeks after amputation. It flattens out slowly into a palm on which four little buds form themselves into digits, although it takes several months for the regenerated hand to grow completely like the one that was cut off (fig. 10). In such experiments the regenerated part is frequently normal, but abnormal forms are not unusual. The ability to reconstitute lost parts is found at all levels of the limb and even the limb-girdle can be extirpated with the limb. To prevent regeneration occurring, it is necessary to remove the spinal column as well at the corresponding level. Results are the same for the hind limb.

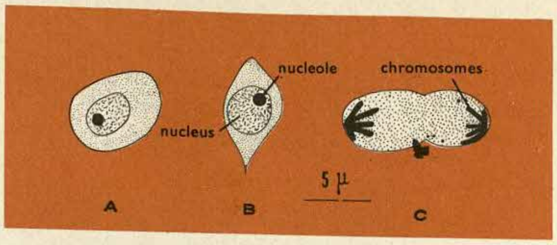


Fig. 7. The so-call regenerating cells are no different from the non-specialised cells of embryos.

Regeneration is necessarily preceded by a general destruction of muscles, bone splinters and cartilage cells which would interfere with healing, to such an extent that the stump no longer exhibits any specialised elements. During this time the cells of the skin actively multiply and collect in a thick pad over the stump. Between the epidermis and the disintegrating tissue the cells of regeneration accumulate. These are typical non-differentiated cells with a large nucleus which are reconstituting the muscles and cartilage.

The newt is a living puzzle

With *Euplanaria lugubris,* we have seen how each fragment can organise the reconstitution of all the lost parts. In newts this ability to reorganise is limited to certain internal regions, called territories.[15] We can best grasp this idea by making a clay model of a planarian. Cut this rough figure in two and the pieces could be remodelled into two small planarians. The newt on the contrary, can be viewed as a many-coloured puzzle within which certain pieces retain a complete plasticity. The front limb and a part of the trunk (all that is shown in the diagram, fig. 9) can produce a front limb but nothing else. All the area shown in blue can produce a tail, etc. The green regions, on the other hand, are quite incapable of regeneration or reorganisation.

This notion of a territory being responsible for a definite piece of regeneration has been demonstrated in several ways. Here is one of these delicate and ingenious experiments which is dependent on the fact that certain nerves of the newt stimulate regeneration. The nerve of the arm is extirpated and then replaced in such a way that its free end can emerge at a chosen point; the skin at this point is periodically removed. When regeneration occurs it is always in the form of a small supplementary hand when the nerve comes

(12) Induction: *Production of one phenomenon by another. For example: regeneration is induced by amputation. The action of one part of an organism on another part to cause it to form a given organ, for example: the brain of a planarian induces certain cells in its vicinity to form an eye.*

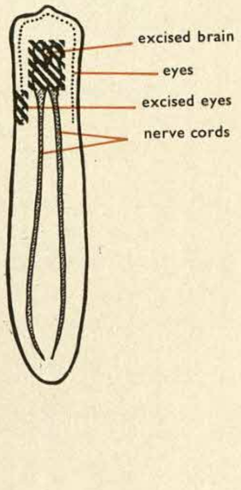
(13) Inhibition: *In embryology and in regeneration, a braking effect on development can go as far as blocking it completely, for example, in a planarian the presence of one head inhibits, that is, prevents, the formation of another head.*

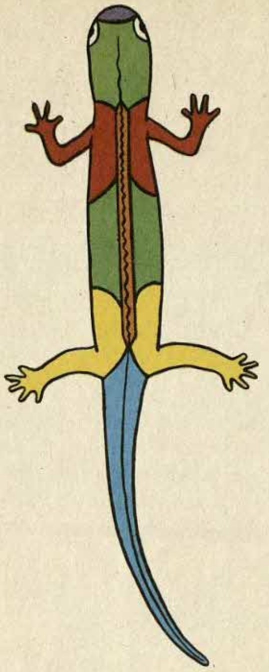
(14) Newts: *Amphibious vertebrates similar to frogs but with an elongated body, short legs and a persisting tail. The most familiar newts in our part of the world are the Palmate Newt, the Common (or Smooth) Newt, the Alpine Newt and the Crested (or Great Warty) Newt.*

(15) Territory: *Part of an organism endowed with particular properties, for example, able to form a given organ or to reform it after destruction.*

(16) *Certain newts have on their backs a little crest which is particularly well-developed in the male at the time of reproduction.*

Fig. 8. The presence of the brain in Planaria *is indispensable for the regeneration of eyes: in fact, repeated excision of the brain prevents this regeneration from taking place.*





HISTORICAL NOTES

1686
The first precise observations on regeneration of organs, by M. THÉVENOT on the tail of a lizard; in 1712 by A. FERCHAULD DE RÉAUMUR on the claw of a crayfish.

1740
ABRAHAM TREMBLEY cut a hydra transversely into two halves; each half reconstituted a complete hydra. From 1740 to 1744 Trembley carried out many experiments and showed notably that two small fragments of a chopped up hydra can join and re-form a normal animal.

1741
CHARLES BONNET verified the fundamental experiment of Trembley on an aquatic annelid worm. Then Bonnet repeated the experiments on an earthworm.

1742
RÉAUMUR discovered regenerative capacity of planaria.

1768
LAZARE SPALLANZANI observed the regeneration of the heads of snails and that of the tail, limbs and front of the head of newts. In the nineteenth century experimenters mapped out more precisely the occurrence of regeneration. Twentieth century research workers have studied the histological constitution of tissues and their innervation, internal factors which prevent, impede or promote regeneration.

out in 'front limb' territory, a small crest[16] when the nerve excites the 'crest' territory. But there will never be any regeneration from the neutral territory: each territory responds according to its specialised function.

A wound heals

Without wishing to make a rigid rule (*Dendrocoelum lacteum* serves us as a reminder) we can say that animals which are not highly specialised have a great capacity for regeneration and that this capacity diminishes as the degree of specialisation and complexity increases; mammals are no longer even capable of regenerating a finger or an ear. However, they have no difficulty with physiological repairs, such as the growth of hair or the renewal of red blood corpuscles. Certain tissues are regenerated: connective tissue, muscular tissue and bone tissue. Finally, the power of traumatic regeneration survives in one organ: the skin. This may not seem much compared with the marvellous capacity of planaria. Yet one should not underrate the importance of this phenomenon: if the skin, which is continually exposed to the assault of external forces, could not regenerate, our chances of survival would be poor. The healing of a wound in planaria may be a simple phenomenon, but the same does not hold for the human skin with its anatomical complexity.

Here is a broad outline of the healing process of an open wound, such as a cut. A clot is formed which obstructs the blood vessels and stops the bleeding. At some distance from the wound (not touching it) the cells of the generative layer of the epidermis and the underlying cells of the living layer multiply, increase in size, and migrate towards the site of the wound. They mass together, forming an epidermis which is often thicker than the normal one, and push off the necrotic tissues, which fall away. The new epidermis in turn peels off its top cells, thus regaining a normal thickness. The dermis then in turn repairs itself beneath the epidermis. The blood capillaries dilate and the connective tissue swells This is the period of inflammation. New capillaries appear and around them the connective tissue cells multiply and form new tissue, which is still poor in elastic fibres. Little by little the dermis acquires more fibres and regains its normal state.

The regeneration of skin is a flexible process: if the skin is not just cut but destroyed over a large area, it is the dermis which regenerates first; the epidermis slides to the surface later. The healing process can be accelerated, for example, by substances which stimulate the multiplication of cells. Surgery is continually making great advances in dealing with extensive wounds.

On several grounds the future seems hopeful in the realm of healing, and possibly even regeneration. Among young frogs which are—after their metamorphosis—incapable of regenerating organs, limb buds have been produced by stimulating the nerve of the limb or by preventing too rapid healing, which seems in some way to suppress the underlying blastema. Also we are beginning to learn more about those cells or tissues which are in a dormant state within the organism, even in man. Such are the dental germ cells of infancy; the reproductive glands, already formed in the embryo but remaining dormant until puberty; such, too, in the adult are certain embryonic connective tissue cells (the object of much research at present), or those fibrocytes which can rapidly revert to an embryonic state when called upon to do so by some sort of trauma. It is not the regenerative material which seems to be lacking in man, and in theory no single tissue in a limb is incapable of regeneration.

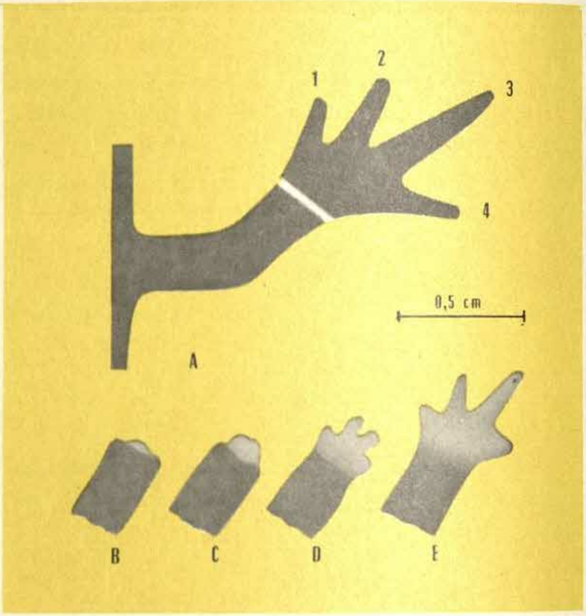
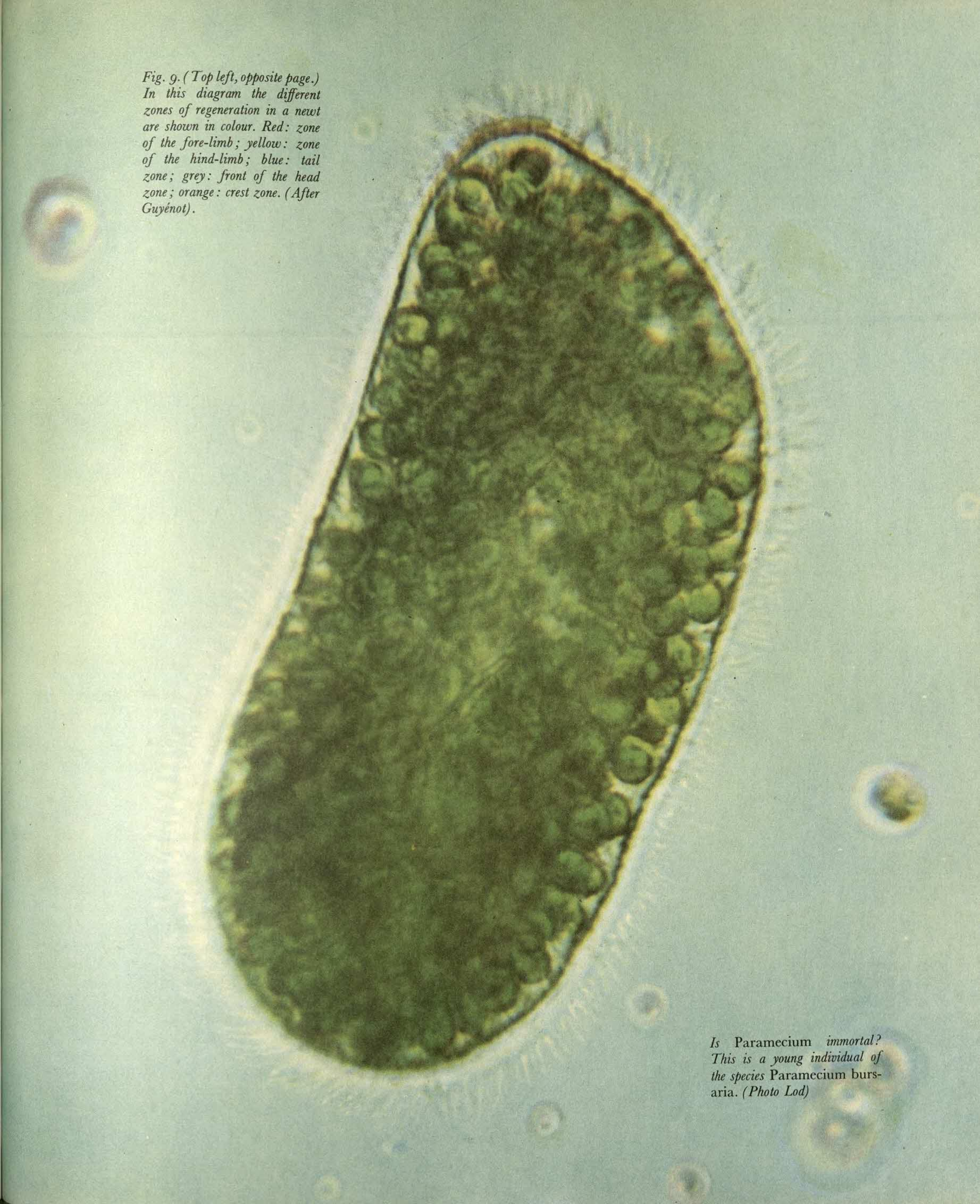


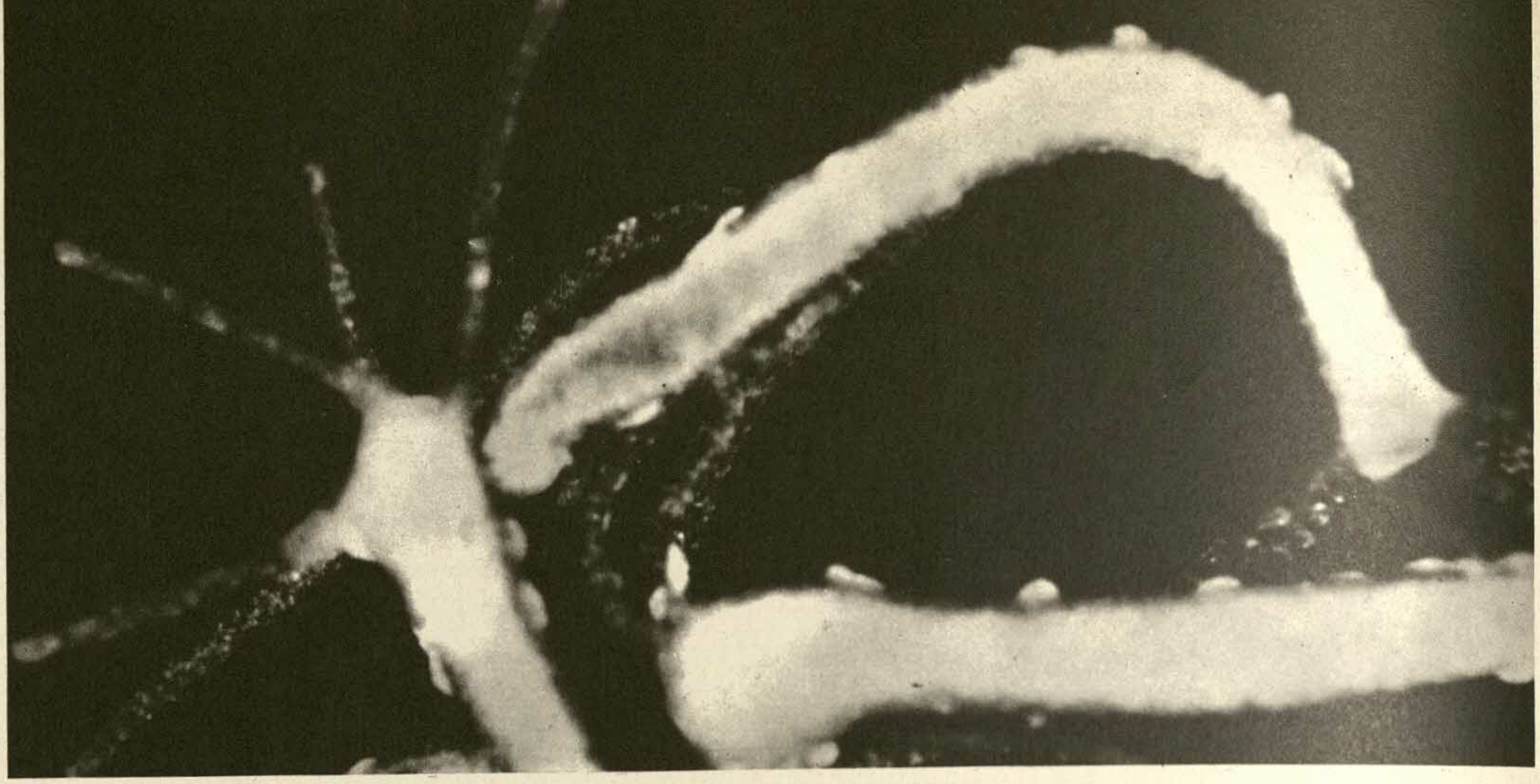
Fig. 10. Regeneration of the hand of a newt.

Perhaps we can end this survey of cells which retain their youthfulness by envisaging the possibility that one day we shall be able to remove the inhibition that rests on the regenerative processes in man, and to achieve—let us be modest—the regeneration of a finger, or even a hand.

Fig. 9. (Top left, opposite page.) In this diagram the different zones of regeneration in a newt are shown in colour. Red: zone of the fore-limb; yellow: zone of the hind-limb; blue: tail zone; grey: front of the head zone; orange: crest zone. (After Guyénot).



Is Paramecium *immortal? This is a young individual of the species* Paramecium bursaria. *(Photo Lod)*



Some animals never die entirely, such as the freshwater hydra (mag. × 15) (photo Lod).

The immortal cells

Paul BRIEN

Death is not inherent in life, since it is the end and negation of life.

Cells taken from an organism and kept *in vitro* in an artificial environment continue to live indefinitely, multiplying incessantly, so long as the culture environment is renewed regularly and the cells are constantly isolated by daily seeding out.

The unicellular protozoa reproduce themselves in immortal lineage in well-kept breeding conditions.

Death only attacks multicellular organisms; this is a direct result of their organisation. Whilst the cells differentiate into tissues and organs adjusted to each other, the necessary conditions for proliferation decrease and are lost. The maturing of the animal also marks the beginning of its progressive and inevitable death.

But some cells in the organism remain young, escaping from this gradual decline, from the encroachment of death.

Germ or sex cells survive the living being which produces them; they perpetuate the species from generation to generation.

Even in the differentiated tissues of the soma, some cells resist ageing better than others. These are the embryonic replacement cells which have the function of helping cells which become exhausted by their differentiation and functions. The ability of an animal to recover or cure its wounds enables it to live for a shorter or longer period. Experimental surgery or medicine become realities, but sooner or later this ability to heal and repair fails. The animal grows old, declines and dies.

Some animals never die entirely because they perpetuate themselves not only by sexual production but also by agamic or asexual propagation, by blastogenesis.[1] Blastogenesis is fundamental to the protozoa; it coincides with cellular division. It is the rule of the vegetable world, and is preponderant among the lower metazoa, sponges, coelenterata, worms, bryozoa and tunicata. Sometimes it alone ensures the survival of the species. In higher metazoa such as the molluscs, arthropoda and vertebrates it disappears and sexual reproduction alone remains.

In the following chapters, we shall examine the case of the paramecium, which is able to divide indefinitely; that of the hydra, which grows continuously; and finally that of the sexual reproduction of higher animals.

(1) Blastogenesis: *faculty of the cells belonging to a localised territory to help to build up a new organism and ensure reproduction without sexual intervention.*

Is paramecium immortal?

Emile VIVIER

The paramecium is a little slipper-shaped organism which is only just visible to the naked eye, being about a fifth of a millimetre long. It belongs to the group of ciliated protozoa known as Infusoria. About a dozen species of paramecia are known and several are found abundantly in the waters of stagnant pools.

Although theoretically a single cell, the paramecium has two nuclei, a macronucleus and a micronucleus both of which are surrounded by a mass of cytoplasm.

Normally the paramecium multiplies by dividing into two, that is to say, by binary fission. First, the micronucleus divides and then the individual, together with its macronucleus, elongates and becomes constricted until finally it divides transversely (fig. 2). The process starts again when each of the individuals has regenerated its missing cell contents and gradually regained its original size. Thus multiplication is ensured by a combination of growth and division. In *Paramecium caudatum*, one of the largest and commonest species, division takes place approximately once every twenty-four hours, while smaller species divide more often. Consequently, the number of individuals in a population doubles each day. However, the growth of the population is usually limited by the intervention of various factors, such as an unfavourable environment or losses due to predatory animals which feed on paramecia and so on.

The special feature of this type of reproduction is that today's children are yesterday's parents. That is, the protoplasm of the descendants is the same as that of the parents, apart from the fact that it has grown. Since these offspring have the same capacity for growth and division, it may be envisaged that under ideal conditions they would never die and that the paramecium, by perpetuating itself indefinitely through its descendants would be immortal.

There is a distinction to be made between the individual and its line of successive descendants. When it undergoes binary fission, the individuality of a paramecium disappears in the process of forming two new individuals. On the other hand, the line may be perpetuated by the indefinite multiplication of its members, thus ensuring the continuity of the protoplasmic basis of the organism.

Paramecium sometimes dies

Nevertheless, if the facts are examined in detail and paramecium is grown in pure culture while more experiments are carried out, the phenomena are shown to be, in reality, more complex than they first appear.

Although, typically, the paramecia continually grow and divide, some of them stop growing, vegetate for a while (usually several days) without dividing, and finally die. Thus the death of an individual can occur even under apparently ideal culture conditions: identical to those under which the other members of the line are able to live. Yet, the paramecia which survive may be regarded as endowed with immortality.

While binary fission is the normal method of multiplication among the Infusoria, the sexual phenomenon known as conjugation also takes place. Morphologically, this is characterised by the coupling of two vegetative individuals (fig. 3). The accompanying nuclear phenomena have been known since 1889, when Maupas carried out his elegant researches, which are now classics among biological investigations.

During conjugation (figs. 5, 1–11), the micronucleus of each of the conjugants undergoes a *reduction division*, which gives rise to two pronuclei, or gametic nuclei, with half the normal chromosome content. One of these, called the migratory nucleus, then crosses the region where the couple are joined together and fuses with the pronucleus, or stationary nucleus, which has remained in the other individual. The conjugants then separate. While the pre-existing macronucleus disintegrates, the nucleus formed from the fusion of the two gamete nuclei divides. The normal diploid chromosome number is restored when the gamete nuclei fuse and the products of this division then give rise to new macronuclei and micronuclei in the individuals which have conjugated according to processes varying from one species to another.[1]

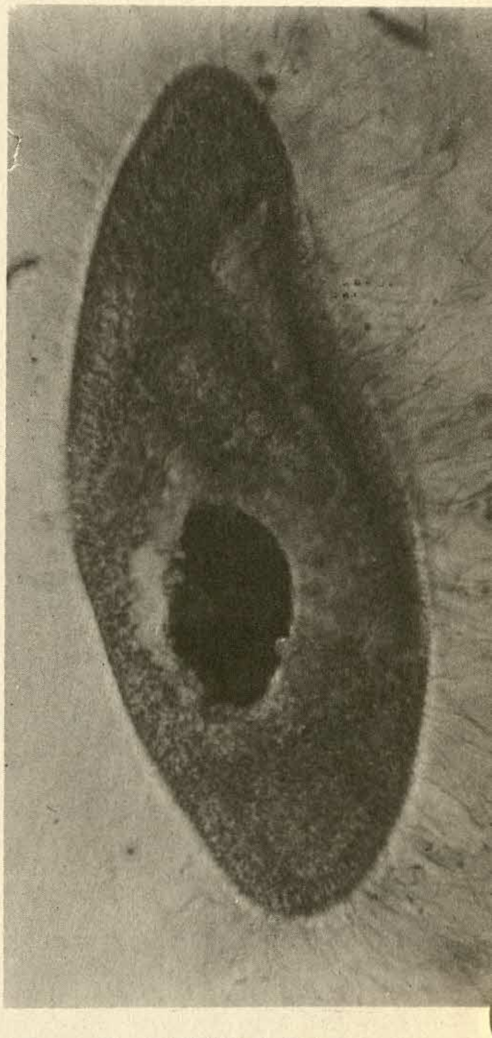


Fig. 1. Single individual of the species Paramecium caudatum *(photo Lod).*

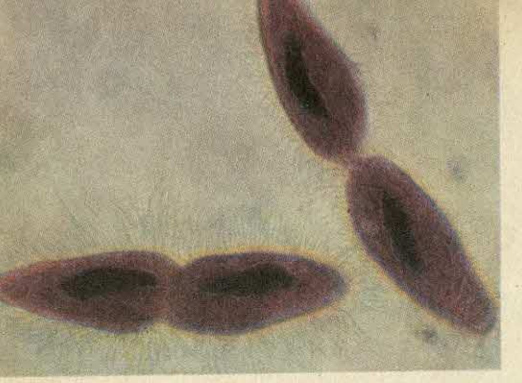


Fig. 2. Dividing paramecia: stained preparation showing the different stages of division (mag. × approx. 250) (photo Lod).

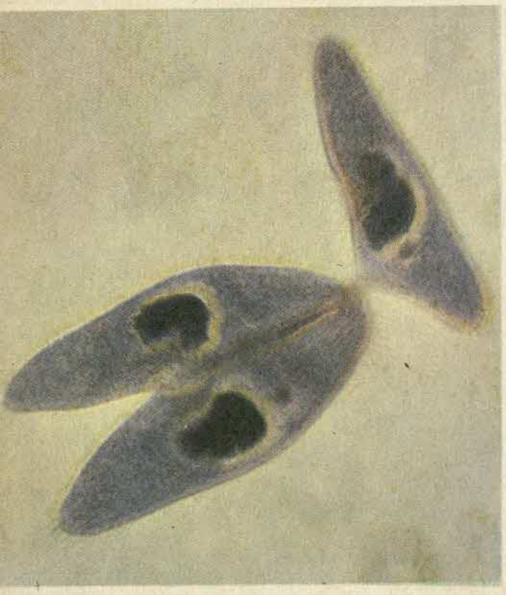


Fig. 3. Conjugating paramecia: a peculiar case of conjugation between three. The individuals which are coupled normally undergo the usual nuclear phenomena. The third, extra, individual undergoes autogamy (mag. × approx. 250) (photo Lod).

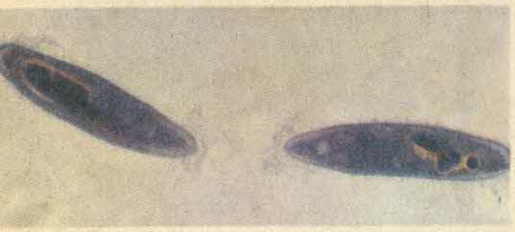


Fig. 4. Paramecia undergoing nuclear changes after conjugation. The fragments of the old nucleus can be made out, and, more clearly, the young ones being formed (mag. approx. × 150) (photo Lod).

The only significant consequence of conjugation apparent on direct observation is a cytological one: the complete renewal of the nuclear apparatus, since in these cases sexual reproduction does not involve any increase in number. The two individuals which have coupled resume the normal mode of vegetative reproduction.

Maupas made careful studies of the development of different species of paramecia in hay infusions and his investigations led him to distinguish three phases during their life:

1. A youthful phase during which the individuals divide actively, but never conjugate, even when in suitable culture conditions. They may be regarded as immature.

2. A mature phase during which the individuals continue to divide normally and, having reached a suitably mature state, they may conjugate. It is only during this period that coupling can be effective. When the conjugants have thus renewed their nuclear apparatus, they can begin another youthful phase (figs 5, I–V).

3. A period of senility which only affects those individuals which have not coupled during the preceding phase. The individuals become smaller, abnormal Infusoria appear more and more frequently and the mortality rate increases, resulting finally in the death of the culture. If conjugations are still possible, they are ineffective and do not produce rejuvenation. Thus, this phase inevitably ends in death.

Therefore, the rejuvenation of a culture by the fusion of two nuclei can only prevent the onset of senescence if it takes place in time. This concept of a cycle of generations seems valid, and is supported by a considerable number of concordant observations. The paramecium, then, like the multicellular living organisms, is not immortal but is enabled to survive on account of its sexuality.

Immortality and autogamy

There was no delay in casting doubt on this striking analogy and the concept of senescence was seriously attacked. Woodruff used a technique of *sub-culturing* the species, *Paramecium aurelia* onto fresh media, without undergoing conjugation. He succeeded in keeping a culture of the same line for thirteen and a half years from May 1, 1907 to December 1920, without its showing any signs of senility. The rate of division was recorded up to May 1, 1915, and was found to be, on average, fifty divisions per month; thus there had been some 8,000 successive generations. Galadjieff and Métalnikoff obtained similar results with the species *Paramecium caudatum*. By isolating the paramecia every day, or every two days, they succeeded in culturing the same line for twenty-two years and 8,704 successive generations without the intervention of conjugation.

Thus, the patient work of these investigators shows that a line of Infusoria may be indefinitely perpetuated without recourse to sexual reproduction; that the phase of senility is not inevitable; and that it is sufficient to place the individuals in appropriate media. These results apparently imply a serious criticism of Maupas's theory, which postulates that a cycle of generations takes place, but further examination of the results should show whether or not they are absolutely incompatable with the original theory.

In fact, investigation showed that there were periodic variations in the rate of division, with alternating periods of depression. If the latter were not fatal, as a result of changing the environment, cytological examination of the paramecia showed that at this time the isolated individuals underwent a nuclear change which took the form of a renewal of the macronucleus from the products of division of the micronucleus.

Certainly the work carried out at that time by Ermann and Woodruff seemed to establish that a new phenomenon took place which acted quite differently from conjugation. But recent studies on the same species indicate that what really takes place is autogamy; this is no different from conjugation, except that coupling does not take place and there is no exchange of migratory nuclei. It consists in the fusion of nuclei which have both arisen from the same nucleus by a reduction division. Thus it is an example of true self-fertilisation which provides the new nuclei after the breakdown of the old macronucleus (fig. 4).

Modern research, carried out on three different species, has brought support to the ageing hypothesis and has provided data on the mechanisms of rejuvenation. Jennings (1944) indicated that *Paramecium bursaria*, shows a period of maturity which lasts several years and then, if conjugation has not intervened, enters a period of decline culminating in many cases, if not in all, in death. During this period of decline, division becomes more infrequent, anomalies appear, many individuals die, and conjugation, if it occurs in old stocks, involves the death of most, if not all, of the conjugants. Sonneborn reported a similar situation in *Paramecium aurelia* (1954–5), and Vivier (1960) found that in *Paramecium caudatum* the hypothesis of ageing is supported by three main arguments:

(a) The loss of the ability to conjugate. When stocks which have been kept in the laboratory for a long time by simple vegetative sub-culturing, are put into the appropriate conditions, they show weak sexual reactions, producing few, if any, conjugations. On the other hand, young cultures just established from ex-conjugants give a strong sexual reaction, producing up to 100 per cent conjugants.

(b) The appearance of nuclear anomalies and their increasing frequency as the stock ages: in old stocks increasing numbers of individuals are found which show anomalies of the macronucleus, such as hypertrophy or *hemimixis*. The latter involves the extrusion of a variable number of small masses of chromatin from the nucleus, or its partial fragmentation.

(c) Conjugation mortality. In young lines such as those coming from individuals which conjugated one or two months previously, conjugation results in a death rate of around 15 per cent in the ex-conjugants or their immediate descendents. In lines which are several years old this mortality is more than 50 per cent, whereas in old lines which show numerous nuclear anomalies, conjugation does not take place under normal conditions, but, if it is experimentally induced, it is followed by the death of all the individuals.

Ageing as a laboratory phenomenon

It has been asked whether ageing, and thus the rejuvenation brought about by conjugation, might not be due to the conditions of laboratory culture. This is an important question but it is not easy to answer, for accurate observations on this point cannot be made in nature and natural conditions cannot be created in the laboratory.

Certain facts lead us to think that ageing occurs in nature. Individuals which have been living under natural conditions, have often refused to conjugate when put under suitable conditions, with other individuals of various mating types.[2] It is reasonable to conclude that they are senile. Also, if individuals removed from natural conditions at the same time are cultured separately, some of the lines are found to show the phenomena of degeneration before others, and to lose the power to conjugate, although they have been reared

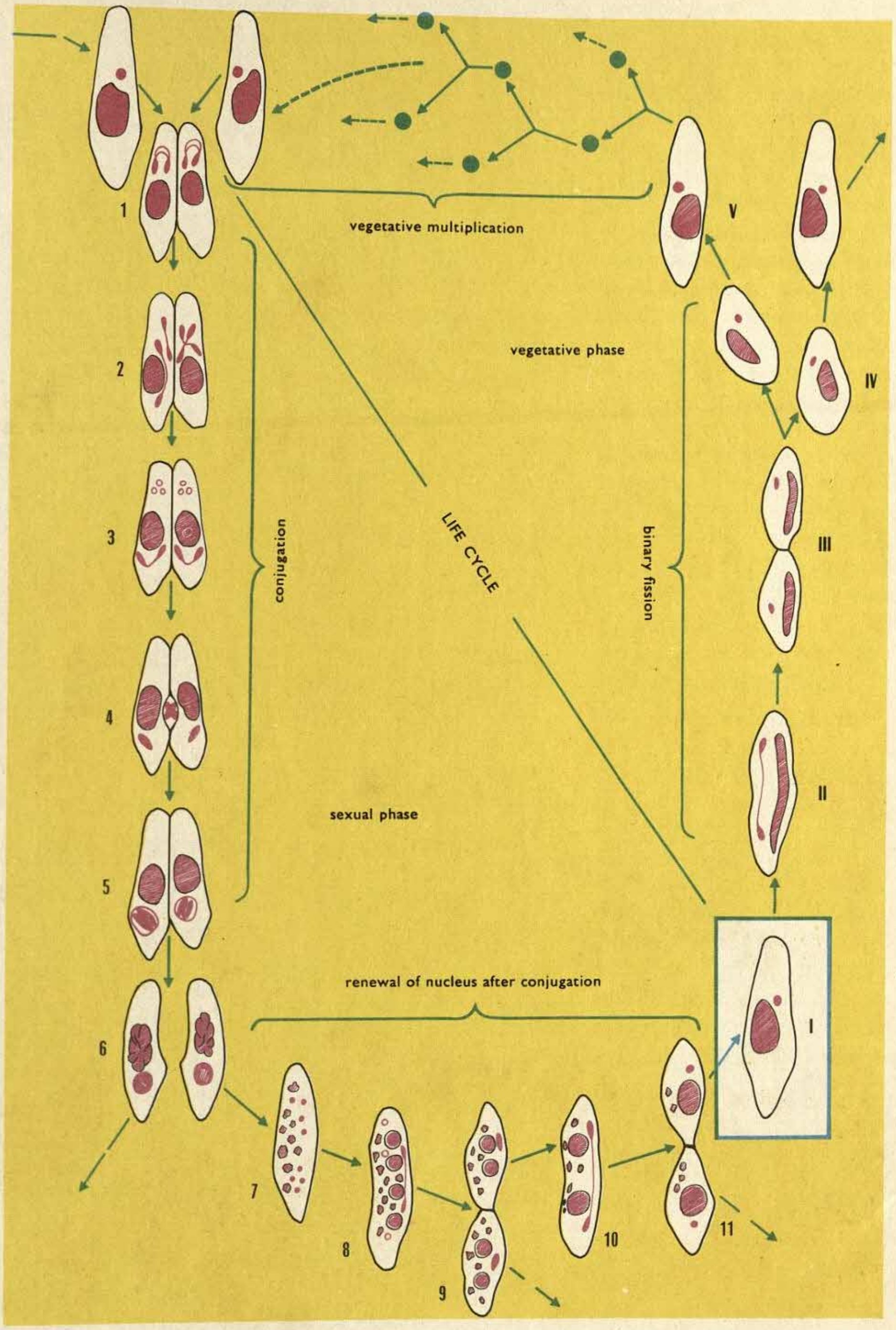


Fig. 5. Diagram of the life cycle of Paramecium caudatum.
1. Beginning of conjugation: first division of the micronucleus.
2. Second division of the micronucleus.
3. Third division of the micronucleus: only one of the four nuclei produced in each conjugant by the preceding phase, undergoes this division (the other three degenerate) and gives rise to the two gamete nuclei: the stationary and the migratory.
4. Exchange of migratory nuclei.
5. Fusion of stationary nucleus with exchanged migratory nucleus: formation of syncaryon.
6. Separation of conjugants; the old macronucleus begins to disintegrate, the syncaryon starts to divide.
7. Beginning of the nuclear changes after conjugation: the old macronucleus is fragmented, the syncaryon has divided into eight nuclei.
8. The fragments of the old macronucleus disappear in the cytoplasm. Four of the division products of the syncaryon develop into four new macronuclei, a fifth divides, the other three degenerate.
9, 10, 11. Nuclear regulation completed by two successive divisions.

I. Young individual with nuclear apparatus entirely regenerated to form the start of a new line.
II. Beginning of vegetative division, showing division of the micronucleus and elongation of the macronucleus.
III–IV. End of binary fission by transverse division.
V. Individual which has resumed its normal size after growth.

under identical conditions. Thus one may suppose that their ageing began in the natural state, since otherwise they should all develop in the same way.

However, it does not follow that the laboratory conditions of culture are without effect on the phenomena under consideration. On the contrary, it is quite probable, and is confirmed by various results obtained by different investigators. The techniques used may shorten or lengthen the different phases in a paramecium's life by a considerable period, and it is not disputed that the conditions of culture, the presence or absence of certain salts in the medium, or the richness and variety of the nourishment provided, may alter the length of the period during which conjugation is possible by several years.

Even if ageing is not due to laboratory conditions of culture, the paramecium remains able to protect itself from ageing by the periodic renewal of its nuclear apparatus. This renewal is normally effected by conjugation, but may also be brought about independently of it. Thus, the secret of the longevity of the organism lies in its nucleus and not in the sexual act itself.

The complexity of paramecium

Numerous observations and experiments have been carried out in experiments which involve cutting the infusoria up into two or more parts in order to separate the nuclei and enable their different functions to be studied independently. These experiments have shown that the macronucleus has essentially a trophic and vegetative function, whilst the micronucleus only functions during reproduction. Deprived of the micronucleus, infusoria may live and divide, but without the macronucleus, life can continue for only a few days or weeks. They do not grow and do not divide. Thus it is the macronucleus which controls a large part of the life of the cell, and it is conceivable that its ageing which, according to cytological examination, brings about various anomalies, may involve correlated anomalies of nutrition, excretion, and so on, thereby leading to the degeneration of the whole cell through the break-down of its essential functions. Conjugation restores new youth to the cell, by effecting the synthesis of a fresh and normal macronucleus within the cytoplasm. But it must take place at the right time during the cycle: when the cell has still enough vigour to tolerate the changes, and before the phenomena of degeneration have destroyed the paramecium's vital capabilities.

The paramecium is a very complex organism, containing many specialised cellular contents. It is even more complex than a simple metazoan cell, and it is possible that it can become tired, poisoned and aged, just as higher organisms do. This situation, therefore, contradicts Weismann's theory which held that unicellular beings (like the germ cells, which are those cells of a multicelluar being which give rise to the embryo and ensure the continuation of the species) are endowed with potential immortality. Thus the macronucleus may be regarded as the equivalent of the metazoan soma or body, when it is destroyed and renewed during each sexual process. That is to say it is the vegetative, perishable, part of the individual. In consequence, the paramecium may be legitimately regarded as being both unicellular and bicellular at the same time. The survival of the surrounding cytoplasm would be due to the periodic renewal of the somatic nuclear body.

Do the conclusions reached by studying paramecia apply to other infusoria? It would be tempting to affirm this. Without doubt the paramecium has been the subject of longer, more careful, and more accurate work, yet conjugation is unknown in many species. For some, especially the smaller ones, have been cultured for many years without showing any periods of depression, conjugation, or the autogamic type of nuclear reorganisation.

According to Fauré-Fremiet, senescence originates in the hyperploidy of the macronucleus, when it contains more than its normal diploid content of chromosomes. This character is supposed to bring with it the irreversible risk of degeneration, particularly, it seems, among the large, highly differentiated species. But, nevertheless, according to this author, this concept of senescence is only a possible and not an obligatory development. The processes of regulation capable of overcoming this risk would be manifested to a varying extent, ranging from the elimination of small masses of nuclear material to the complete replacement of the decaying macronucleus by a new one, by undergoing gradually bigger changes. Thus, this reorganisation would either be small, slow, progressive and difficult to discern, or more extensive and easily observable.

However, in conclusion it may be said that Maupas's old concept of senescence and rejuvenation remains acceptable in its broad outlines, but it needs to be reconsidered and made more flexible in the light of new data.

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(1) Syncarion: *term applied to nuclei after fusion. After conjugation, the syncaryon divides a certain number of times and thus gives rise to 1, 2, or 4 macronuclei, according to the species. These are then distributed between the individuals of the following generations. For example, in* Paramecium caudatum, *four macronuclei are formed, so that the normal number of nuclei per cell is not reached until after the cell has twice divided into two, that is, after the ex-conjugant has given rise to four individuals. In* Paramecium aurelia *only two macronuclei are formed from the syncaryon, so that the first division of the cell brings about nuclear regulation.*

(2) *Mating types: individuals of a certain strain have an affinity for individuals of another strain, called the opposite or complementary mating type, which leads them to conjugate together. These strains are morphologically identical but sexually differentiated. Each species of infusoria carries a certain number of sexually distinct varieties, each including two or more complementary mating types.*

The freshwater hydra

Paul BRIEN

Asexual reproduction takes place in a variety of ways in the animal kingdom. Whatever pattern it follows—and this depends on the structure of the organism concerned—it consists in the formation of new individuals from multicellular fragments which separate off from a blastogenic zone belonging to a localised area of the body. This is the process known as budding. In the blastogenic zone, in which growth is constantly taking place, the cells appear to elude the compulsion, control and inhibition exerted by the organism as a whole;[1] they are, as it were, in a state of physiological isolation. Although they belong to the body, or soma, these somatocytes[2] remain in (or return to) an earlier embryonic state. They may undergo dedifferentiation or remain undifferentiated; they become rich in ribonucleic acids, increase somewhat in size and acquire a great capacity for proliferation.

Their multiplication might, in its early stages, appear similar to tumour formation. Yet it is never abnormal and never anarchical; on the contrary, it leads to specific organogenesis. Thus the cells in blastogenesis recover the ontogenetic faculty[3] of the fertilised egg. Their constant division leads to the formation of a new organism, similar to the parent, and showing strict transmissibility of specific characteristics and remarkably stable somatic heredity.[4]

Successive generations of buds sometimes remain together in colonies, but often they disperse, thus making wider propagation possible. They are highly resistant to the most unfavourable conditions, including cold and dryness. In the sponges, the bryozoa and the tunicates, there are winter buds which ensure the continuity of the species from year to year.

In certain animals, therefore, part of the soma is endowed with immortality. For the entire living creature to be immortal, however, it would have to be continuously growing and possess the faculty of constantly renewing its elements and repairing, everywhere and all the time, the wear and tear or deterioration resulting from its metabolic processes. Such creatures exist; and the most striking example known at present in the animal kingdom is the freshwater hydra.

This is a small polyp occurring in large numbers in our streams, rivers, canals and ponds, where it is represented by a variety of species, several of which are quite common: *Hydra fusca* (*Pelmatohydra oligactis*), *Hydra attenuata*, *Hydra viridis* (*Chlorohydra viridis*). Apart from a few variants, these species have the same structure and behave in the same way. The body is slender and flexible, and consists of a hollow cylindrical cavity about one centimetre long. It attaches itself to any solid material underwater by means of a disc or foot,[5] pierced by a small opening. At the other end there is a cone or hypostome pierced at the top by the mouth, narrow, generally shut, and encircled by a ring of long, hollow, sinuous and sensitive tentacles.

Continuous budding, indefinite growth

Observation of the freshwater hydra in isolated culture in water which is constantly renewed, where it is regularly supplied with food and kept at a temperature of 19°C, shows that it exists in a state of unfailing youth, avid for nourishment, and always remains capable of propagating itself by uninterrupted budding.

Other things being equal, the rate of production of buds corresponds to the rate of metabolism and growth. Budding is continuous because growth is unlimited.

The blastogenic zone of the hydra is situated at the base of the gastric tube where digestion is most active; the buds make their appearance in the form of small bulgings of the body wall, which, within two or three days, become organised into young polyps. A well-nourished hydra generally shows several buds

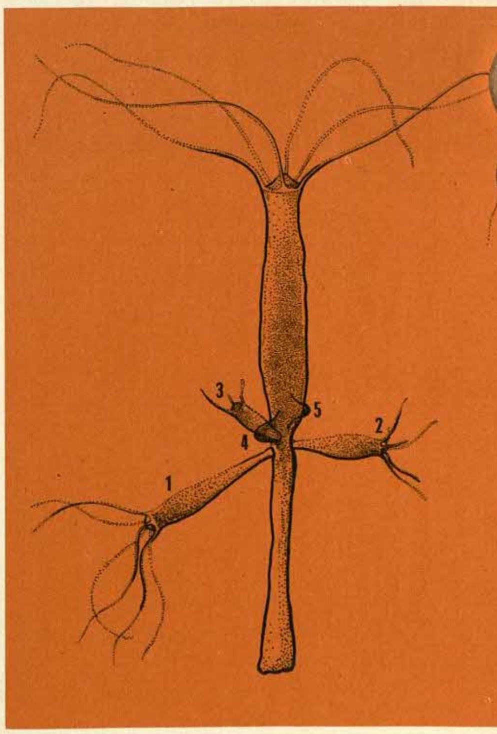


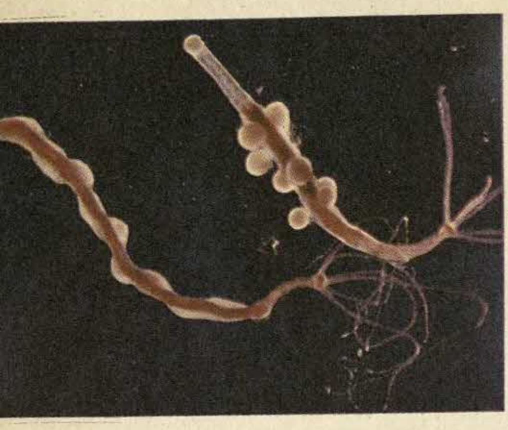
Fig. 1. Diagram of Hydra oligactis *to show the appearance of the polyp and of its parts; the ring of tentacles round the base of the hypostome and the mouth at the tip of the hypostome, the gastric cavity (with the buds arising at its base), the peduncle and its adhesive basal disc or foot. The buds (numbered 1 to 5) are arranged in order of age: the youngest (5) is the highest; the oldest (1) is attached at the level of the peduncle and ready to separate from the parent.*

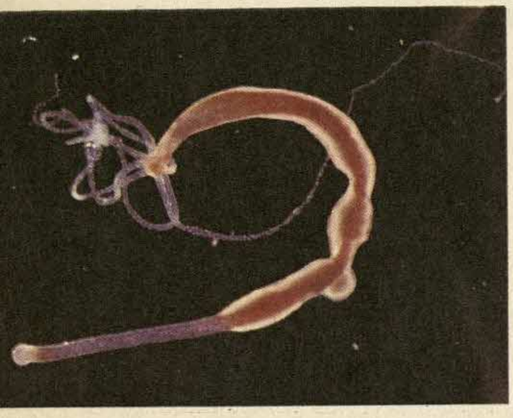
(1) Coercion: *In a multicellular organism a close correlation is established between the constituent elements. This correlation, by exerting a restraining influence on cell activity, ensures co-ordination between structure and function, thus providing the adjustment needed to maintain the morphological and physiological unity of the living creature.*

(2) Somatocytes: *Cells which make up the differentiated tissues of the body, or 'soma', of the living organism—muscle cells, nerve cells, gland cells, etc.*

(3) Ontogenetic faculty. *The faculty of forming a new individual by embryonic development.*

(4) Somatic heredity. *Transmissibility of the characteristics of the species by asexual reproduction through the bud cells, which derive from somatic tissue and not from germ cells.*





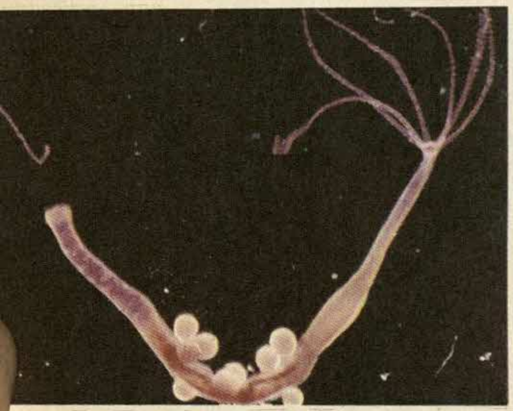


Fig. 2. Hydra oligactis. *Male and female in gametogenesis. On the male (left) are seen the testicular swellings and on the female (right) ova already freed. Mag. c. 5.*
Fig. 3. Hydra oligactis. *Female at time of formation of ovarian swelling in the thickened ectoderm. Mag. c. 10.*
Fig. 4. Hydra oligactis. *Female during emergence of ova from the ovarian swellings in which they were formed. Mag. × 10 (all three photos Brien).*

which have appeared one after the other. The youngest arise on the top of the blastogenic zone, while the oldest, by the time they are ready to separate, occupy the lower part at the level of attachment of the foot. The buds of successive generations are thus arranged in a spiral sequence, and it would therefore seem that they move downwards as they mature, leaving room above for the younger ones to appear and descend to the foot where they become free. This movement is the result of the growth of hydra, which can be defined and localised (fig. 1).

Sometimes a supplementary tentacle develops slightly below the normal ring of tentacles, diverges from it a little more each day, and soon appears on the side of the gastric cavity to reach the blastogenic zone. If it persists as far as that, it disappears only when it reaches the basal disc.

Hydra fusca is gonochoristic[6] (figs. 2, 3 and 4). Ovarian swellings[7] appear within the central gastric cavity and in each of them an ovum is formed and remains enclosed in a fold of ectoderm like an acorn in its cup. As soon as it is fertilised, it undergoes segmentation and secretes a protective case, the oötheca. Sheltered in this, the embryo sinks to the bottom of the pond and there continues to develop. Although the ovum during the initial stages of its development is still attached to the body, it does not remain stationary, but 'descends' further every day until it reaches the level of insertion of the foot, which is the point at which it drops off from the parent. The cup in which it was contained is then visible as a scar; it takes some time to move along the peduncle and disappears only at the basal disc.

Many observers have reported the ability of the hydra to split longitudinally to form two hydras by axial fission although what in fact happens is neither fission nor longitudinal division. The hypostome of a hydra not infrequently becomes doubled, so that two mouths take shape, each surrounded by its own ring of tentacles. The hydra now has two heads and therefore two zones of growth. Each head 'builds up' its own cylinder on a common base, which becomes increasingly slender. The result is a double hydra formed of two gastric regions, each provided with its hypostome and its ring of tentacles; they arise in diverging directions from a single peduncle which becomes progressively smaller and finally disappears. The twin hydras then separate from each other.

These findings indicate clearly that there is a frontal sub-tentacular zone of growth and that any given point of the hydra will occupy successively lower levels, from the anterior part of the cylinder to the basal disc. The time required for this downward shift is three to four weeks; in other words, after a month a new hydra has formed.

So great is the capacity for healing and regeneration, that if two sections, each taken from a different individual, are strung on to a platinum wire they will fuse to reconstitute a new and normal hydra. If before the operation one of the two individuals is coloured with a vital stain such as Nile blue A (or neutral red), the new hydra will consist of two differently coloured portions, the anterior portion which bears the tentacles being blue and the peduncular portion of normal colour, or *vice versa*. If it is the anterior section that is coloured, observation over the next four or five weeks will show that the blue anterior portion of the new hydra, which contains the growth zone gradually replaces the normally coloured posterior portion, which, having no growth zone, becomes smaller and eventually disappears. If, on the other hand, the anterior section is left in its normal colour and the posterior section stained blue, the opposite will be the case.

When a ring of tissue taken from a coloured hydra is inserted between two sections of a normal hydra, it is seen to occupy successively lower levels. It gradually approaches the blastogenic zone, becomes peduncular and finally disappears at the basal disc, by which time the whole hydra has recovered its normal colour (fig. 5).

One might expect the presence of a zone of growth in the upper part of the gastric cavity to result in indefinite elongation of the polyp. Yet in fact its length varies only within the limits of its contraction and extension. This is because the growth potential is absorbed by the formation of the tentacles and, above all, by the continual emission of buds which separate off as soon as they are formed. It is also neutralised in a third way. Observation of hydra shows that the basal disc becomes surrounded by a crown of disintegrating tissue

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(5) Basal disc: *Foot of the column of the polyp in contact with its attachment. It is a highly glandular adhesive base, perforated in the centre by a small orifice, the aboral opening or aboral pore.*

(6) Gonochoristic: *A species in which the sexes are separate is called gonochoristic or dioecious (i.e., the polyps are either exclusively male or exclusively female), in contrast to hermaphrodite species in which the same individual is both male and female.*

which makes it necessary for the breeding tanks to be repeatedly cleaned to prevent their invasion by bacteria. This ejection of dead tissue is a consequence of the peculiar physiology of the peduncle, which lies beneath the blastogenic zone (where the tissue of the body wall is engaged in the constant formation of buds) and takes no part in digestion. Its metabolic rate is low and it becomes literally worn away at the basal disc. Plugs of disintegrating endodermal cells are continuously being evacuated through the aboral pore,[8] while the ectodermal cells are used up in their glandular function and degenerate in the sticky mucous secretion.

An example of a mutant hydra

In the course of prolonged cultures of green hydra a new type has appeared, the special feature of which is that the peduncle or foot does not taper off but, on the contrary, keeps the characteristics of the gastric cavity; it elongates indefinitely, at the same time becoming segmented into tiny cylinders or 'stolons'. Each stolon has retained the blastogenic property characteristic of the column and, like the latter, gives rise along its side to one or two polyps. All the buds, whether arising from the normal blastogenic zone of the cylinder or from the individual stolons, produce stolonate hydras exclusively and with absolute constancy. This is an example of 'bud mutation' or 'somatic mutation', since the ova of these stolonate hydras when fertilised give rise to typical hydras only.

These normal and stolonate green hydras lend themselves readily to grafting experiments of the type described, in which two sections, each taken from a different individual, are joined together to form a single one. Grafts of the anterior part of a stolonate hydra on the lower section of a normal hydra result in three or four weeks' time in a wholly stolonate hydra. The opposite result is obtained if the anterior section belongs to a normal hydra and the lower section to a stolonate one. In both cases the anterior section, which contains the growth zone, elongates until it has reconstituted an entire hydra of its own original type; this new hydra inevitably becomes substituted for the lower section which has no growth zone and is bound to disappear.

The interstitial cells

Histological study of hydra reveals the cellular framework within which these processes of continuous growth take place (fig. 6). The two epithelial layers of the body wall are not composed solely of musculo-epithelial cells; scattered among the ordinary epithelial cells of the ectoderm are a number of sensory cells, at the base of which lies a nerve net. The ectoderm is also packed with developing or fully developed nematocysts[9] which are mainly functional on the tentacles, towards which they migrate. Also lying between the cells of the ectoderm are the interstitial cells which are basophil,[10] rich in ribonucleic acids, proliferative and capable of differentiating in any direction. They are reserve cells which have remained in an embryonic state, capable of self-differentiation at any time into any of the other kinds of functional cell; they can even become, *inter alia*, glandular cells.

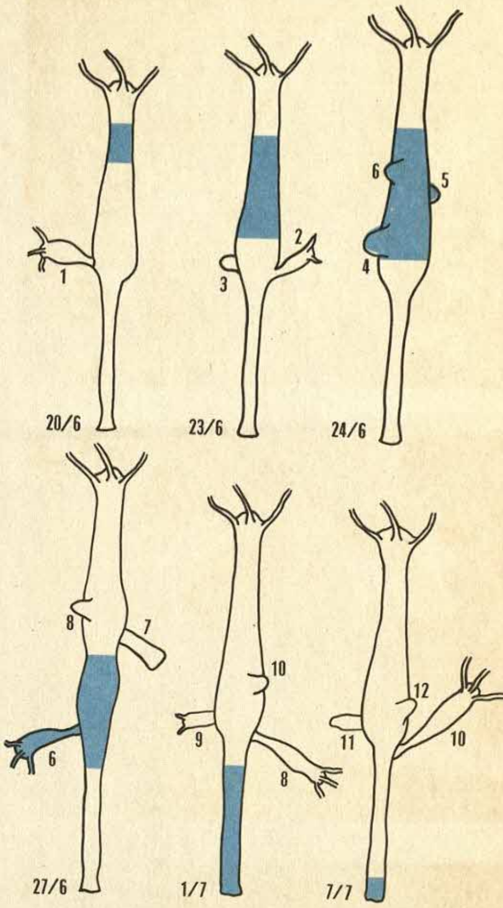

Fig. 5. Diagram of a grafting operation. A ring of tissue taken from the hydra stained with Nile blue has been grafted between two sections of a normal hydra. The graft has taken successfully. The blue ring can be seen moving down the cylinder and the peduncle, to be ejected finally as the basal disc. (Experiment June 20 to July 7.)

These interstitial cells are utilized especially for the formation of nematocysts. This is a constant process, replenishing the stock of stinging organs used to capture prey.

The interstitial cells are thus the important elements in the growth of the hydra. They represent the neoblasts,[11] or polyvalent somatocytes, and are present in the upper area below the mouth, where their rate of division appears to be the highest and where observation and experiment have localized the growth zone. They are particularly abundant in the cylinder of the polyp, and it is here that they produce the inexhaustible supplies of differentiating cnidoblasts.[12] On the other hand, they are sparse in the ectoderm of the peduncle, perhaps because they have been absorbed by the intense blastogenesis. Neither ectodermal nor endodermal cells grow anew in the peduncular region and the peduncle ends in its basal disc.

Thus histological study displays the processes of growth and of wear and tear which, by neutralising one another, ensure constancy of structure, appearance, physiology and persistence in each individual organism.

The interstitial cells are the precursor elements from which the various types of cells are formed and differentiated and without them neither growth nor budding would be possible. If they disappeared or diverged from their normal histological functions, growth would cease and the death of the hydra inevitably follow. They can, however, be destroyed by irradiation with x-rays without the other differentiated cells being affected. During the first ten days of irradiation the

(7) Ovarian swelling: *Term used for the bulge in the ectoderm of the cylinder of the polyp, beneath which the ova are formed.*

(8) Aboral pore or opening: *An orifice in the middle of the basal disc or foot which adheres to the substratum.*

(9) Nematocysts: *Small stinging organs present in cells called cnidocytes and situated in the ectoderm.*

(10) Basophil: *Cells are called basophil when their cytoplasm, becoming rich in ribonucleic acid, stains with the basic dyes used in histological technique, e.g., haematoxylin.*

(11) Neoblasts: *Cells which have remained young and embryonic and capable of differentiation into different types of cell and tissue according to the animal's physiological needs.*

(12) Cnidoblasts: *Cells in the ectoderm of a polyp which form the nematocysts.*

(13) Gametogenesis: *The formation of the reproductive cells (germ cells) known as gametes (ova and spermatozoa).*

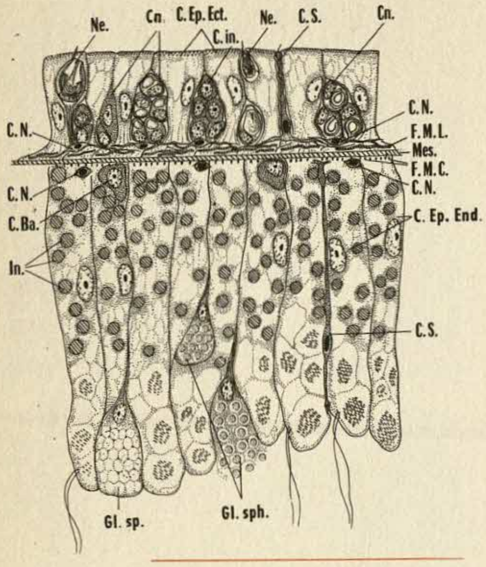

Fig. 6. Section of portion of body wall of a hydra showing the two layers, ectoderm and endoderm, separated by a structureless lamella or mesogloea. C.Ep.Ect.: *ectodermal epithelial cells;* C.Ep.End.: *endodermal epithelial cells;* Mes.: *mesogloea;* C.in.: *interstitial cells;* Cn.: *cells forming the nematocysts;* Ne.: *formed nematocysts;* C.S.: *sensory cells;* C.N.: *nerve cells;* C.Ba.: *basal embryonic cells of endoderm;* In.: *granules of digestive secretion;* Gl.sp.: *spumous gland cells;* Gl.sph.: *globular gland cells;* F.M.L.: *longitudinal muscle fibres;* F.M.C.: *circular muscle fibres.*

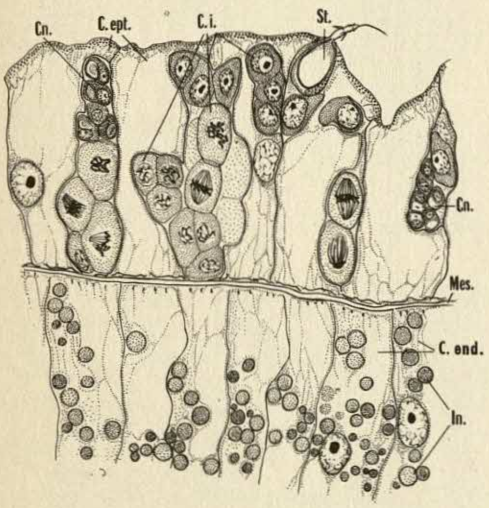

Fig. 7. Section of portion of body wall of Hydra attenuata *at beginning of gametogenesis. Lying among the large vacuolated epithelial cells of the ectoderm* (C.ept.) *and their base, interstitial cells* (C.i.) *are undergoing intensive division and transformation into germ cells. They no longer undergo differentiation into nematocysts* (Cn., St.). *The nematocysts, which were formed before the gametic phase began, are pushed out towards the periphery, where they will be dissolved. Only the oögonia or egg-mother-cells, will remain.* Mes.: *Mesogloeal lamella separating ectoderm from endoderm;* C.End.: *gastric endodermal cells with granules of digestive secretion* (In.).

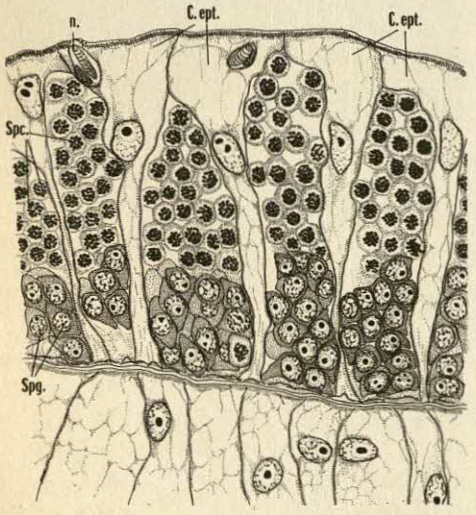

Fig. 8. Section of portion of the body wall of Hydra attenuata *during spermatogenesis, at the level of a testicular protuberance. Lying among the vacuolated and markedly distended ectodermal epithelial cells* (C.ept.), *the basal interstitial cells are developing into spermatogonia* (Spg.) *and these latter into spermatocytes* (Spc.), *which will shortly become spermatozoa. On the other hand, no more nematocysts are being formed. In this section a few nematocysts* (N.), *differentiated before gametogenesis began, are still present towards the periphery. They will eventually disappear. The hydra will then have used up all its interstitial cells exclusively in spermatogenesis.*

hydras behave normally, feed and bud. They then become pale, regurgitate necrotic endodermal tissue through the mouth and stop taking nourishment. When the tentacles, deprived of cnidoblasts (which can no longer be formed) become unable to capture prey, the hydras diminish in size, take the shape of small translucent masses and disintegrate. Loss of their interstitial cells has killed them.

If, however, before the regression has gone too far, the dying hydra receives a graft of a section of healthy hydra, it will certainly survive. The interstitial cells of the healthy fragment migrate into the sick, irradiated ectoderm, re-populate it and reconstitute all the elements essential for normal life.

Gametogenesis

The result is the same if the interstitial cells are turned aside from their somatocyte function (figs. 7 and 8). Certain species—*Hydra fusca, littoralis, pirardi*—enter into gametogenesis with remarkable synchronism if kept at 8°c; the germ cells are formed from the interstitial cells. Gametogenesis[13] is a physiological crisis in which the interstitial cells are diverted from their functions as somatic histoblasts[14] to become germ cells. By monopolising the interstitial cells, gametogenesis deprives the hydra of the basic elements it needs for growth. When, under artificial conditions, gametogenesis extends to the whole cylinder and is maintained—as happens at 8°c for the species in question—the hydra gives up all its interstitial cells and exhausts its substance in producing spermatozoa and ova. It then suffers the same fate as the irradiated hydra—stops budding, and inevitably dies.

On the other hand, if the temperature of a hydra in which gametogenesis was started at 8°c is subsequently raised to 19°c, the sexual phase stops immediately. The interstitial cells resume their initial functions and become once again somatic histoblasts; the hydra reconstitutes its cnidoblasts and its nematocysts and reverts to its normal behaviour, its growth, its budding and its perenniality.

The permanence of individuality

Sexuality is not a part of the biological cycle of every animal, since certain species of hydra never show it at 19°c, although continuing to grow and to reproduce by budding. Sexuality is an epiphenomenon of growth. It is not predetermined, but is determined by a chain of contingent external factors and of internal factors upon which the creature's entire general physiology depends.

The germ cells have no other origin than the cells which will form the soma. More precisely, the soma cannot be regarded as an outgrowth of the germ cells or as its nutritive envelope destined to die and to be renewed from generation to generation. It is in it and by it that the cells, which will give rise to the sex cells, sooner or later become differentiated and that gametogenesis begins.

There are present in animals cells which retain their youth. In the hydra this reserve of young, embryonic cells is represented by the interstitial cells, which are polyvalent. According to the need of the organism, they are the somatic histoblasts of continuous growth or, during the gamete phase, they engender the germ cells.

The hydra is so organised that the results of tissue wear and tear can be repaired at any time. It is immortal; its 'individuality' survives in its specific characteristics, although its constituent elements are constantly being utilised and constantly being replaced.

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[14] Somatic histoblasts: *Embryonic cells destined to become differentiated into the different tissues of the body or soma: eg, ectodermal, endodermal, muscle, gland, and nerve cells.*

The germ cells

Pierre LAVIOLETTE

One of the most important problems that living things have to solve in order to survive is how to perpetuate their kind. They must reproduce or become extinct: they face no other alternative. Consequently all organisms, particularly those belonging to the animal world, have remarkably effective ways of reproducing themselves. These methods are extremely varied, but can, nevertheless, be grouped into two distinct categories:

Asexual reproduction

This takes place without gametes and is very widespread among the lower organisms, where it is sometimes completely exclusive. It can be directly related to all that is known of the capabilities of the living cell for proliferation, differentiation and regulation; for reproduction to be successful, all that is necessary is the capacity to break up into pieces, each of which is endowed with sufficient regenerative power to reform new individuals. After division, the original individual ordinarily replaces the parts it has just lost. Such processes are extremely frequent among the protista, where a single parent can give rise to many successive generations.

Sometimes animals on a higher level of organisation have kept this ability, which makes a sporadic appearance among sea anemones and some annelid worms. In the latter group there is even a marine annelid which reproduces asexually so effectively that its body separates into as many parts as it has segments and each of these pieces can develop into, not one, but four new worms.

Finally, we will mention the very peculiar phenomenon called polyembryony,[1] which may be regarded as an extremely precocious type of asexual reproduction, where one egg gives rise to more than one organism. Certain insects are the record-holders in this respect. For example, among some of the entomophagous (insect-eating) Hymenoptera, a single egg gives rise to one embryo, but this then breaks into a hundred or more fragments each of which develops into a normal larva. It is not until we come to the mammals that we find animals capable of comparable achievements. The South African armadillo and man are akin in this respect, since both can give birth to a series of identical twins (four or eight in the armadillo, two to five in man) arising from one egg (figs. 1 and 2).

Sexual reproduction

Sexual reproduction necessitates the specialisation of an organism. The primary characteristic of this kind of reproduction is the existence of specialised cells, the gametes, which unite to produce a fertilised egg, or zygote. Among higher animals the degree of dimorphism[2] (difference in morphology) between the gametes of the two sexes is extremely marked. The male gamete, or spermatozoon, is often several hundred thousand times smaller than the female gamete, or ovum. However, among lower organisms the contrast is much less obvious and isogamy (gametes having identical morphology) is common. In spite of these differences between the relative sizes of the gametes found in each species, the rule of the pairing of the gametes remains constant throughout, apart from the well-known exception of parthenogenesis.[3]

One result of fertilisation is that it is inevitably accompanied by a doubling of the set of nuclear chromosomes and, in consequence, just as inevitably necessitates the intervention of a reduction division at a particular time. This chromosome reduction which compensates for fertilisation by halving the number of chromosomes, may occur before fertilisation in some cases and afterwards in others. Thus this alternation of the nuclear cycle is one of the surest criteria of sex.

One of the additional consequences of sexual reproduction is the morphological

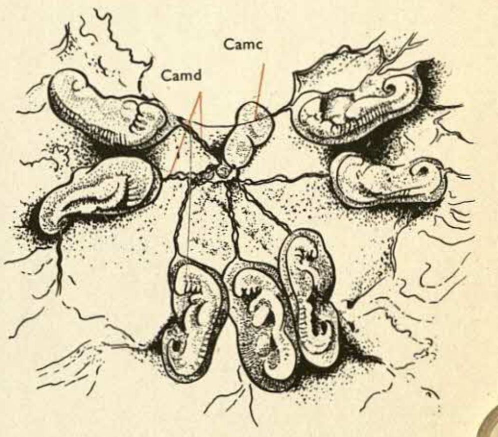

Fig. 1. Seven uniovular embryos of an armadillo (Dasypus septemcinctus). *Camc: common amniotic cavity. Camd: amniotic ducts. These seven embryos have arisen from a single egg. They are identical 'twins'. (After Grassé).*

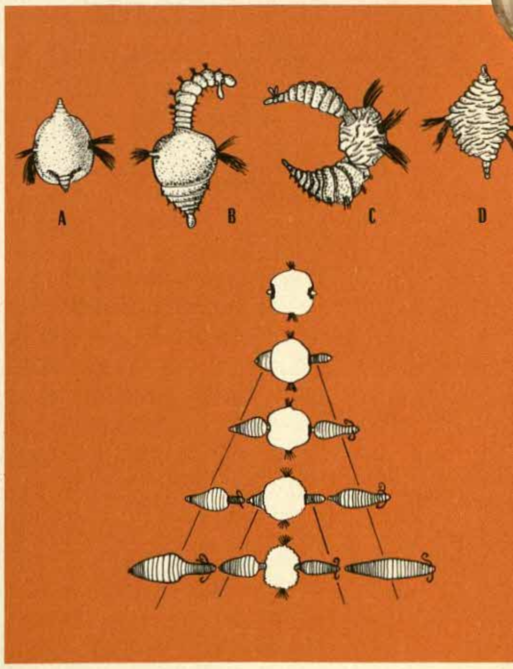

Fig. 2. Schizometamery in Dodecaceria caulleryi. *(a) Metamere beginning to bud. (b) Schizozooids further developed. (c) The two schizozooids are ready to break away. (d) The original metamere, now wrinkled, begins to produce two further schizozooids of the second generation. Below, a diagram of the process of schizometamery. (By this term is meant the budding of an isolated metamere or segment, the animal, an annelid worm, having first split up into individual segments). (After Grassé).*

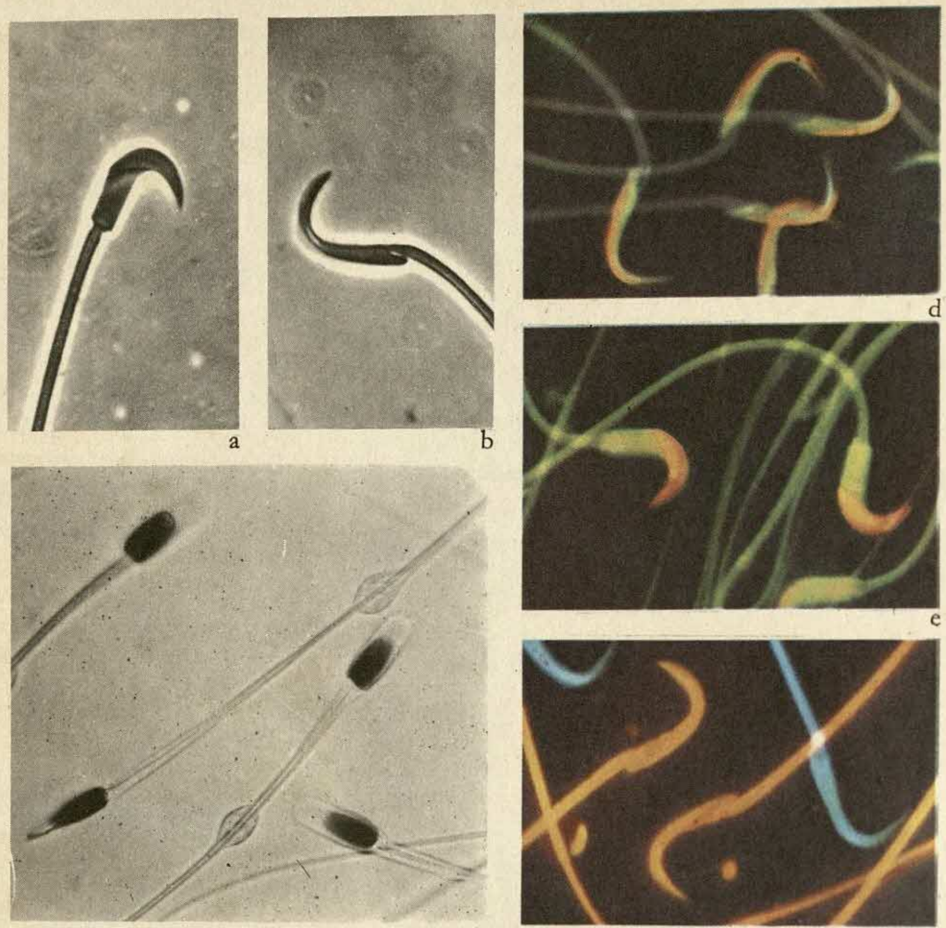

Fig. 3. (a) and (b) Living spermatozoa seen under the phase-contrast microscope. (a) golden hamster. (b) albino rat (mag. × 2000). (c) Spermatozoa of the bat seen under the ultra-violet microscope. One can see that there is practically no absorption at the very tip of the head of the sperm, the acrosome (mag. × 4000).
(d), (e) and (f) Mammalian spermatozoa seen under the fluorescence microscope. (d) Living spermatozoa of the albino rat. (e) living spermatozoa of the golden hamster. (f) living (yellow) and dead (blue) spermatozoa of the rat (differential fluorescence) (mag. × 660) Photo, Bishop and Austin).

expression of sexual bipotentiality: that is, the existence of two distinct sexes within a species, each of which is equipped with gonads and a number of more or less well-defined or secondary sexual characteristics (figs. 3 and 4).

Determination of the sexual state

We have already seen that sex may appear in some organisms almost as though by accident.[4] This point may be illustrated by a few examples.

It has long been known that among unicellular organisms, and especially in the group Phytomonadina, sex may be determined by a variety of physical and chemical factors. For example, it is often sufficient to dilute the normal culture medium, or to deprive it of one particular substance in order to initiate a phase of sexual activity, which is shown by numerous pairings in organisms which have up till then reproduced by solely asexual means. The onset of sexual reproduction may be similarly brought about by varying the acidity of the culture, the temperature or the concentration of an ion, such as calcium, and even light has usually been found to be an indispensable environmental condition. In one particular case it has actually been possible to obtain an active chemical principle from a sexually active culture which, when added to a culture without any tendency to reproduce sexually, causes it to enter into a phase of gametic activity. Evidently a sex-stimulating substance is at work, but its exact nature is still uncertain.

Another example is that of various Protozoan parasites which do not become sexually active until the time when their host undergoes metamorphosis. During the larval life of the latter, the parasites multiply by strictly asexual means and it can be inferred, although it has not been proved, that the hormones responsible for bringing about metamorphosis in the host may be the determinants which initiate the sexual phase of the parasite.

These two modes of reproduction which are found together so frequently in the Protozoa are also found to exist concurrently in some groups of multicellular organisms. The few examples we have just described have shown that, contrary to widespread belief, there is no necessary connection between sexuality and reproduction. Naturally, among higher animals it cannot be denied that sexuality is of the greatest importance, but taking the animal kingdom as a whole, this is less true. Among lower organisms the sexual mechanisms, at the same time so individual and so universal, only appear from time to time. The influence of environmental factors, of overwhelming importance in the lower orders of the animal kingdom, gradually become less and less so higher up the evolutionary scale. Does this mean that the highly specialised reproductive processes of the higher animals are entirely free from environmental influence? We shall see that this is not so, although it acts on a different phase, namely sex determination.

Sexual lability

Earlier we draw attention to the commonplace concept of the existence of two complementary sexes within each species. Usually the numerical balance, or sex ratio,[5]

(1) Polyembryony: *The origin of many organisms from a single egg.*

(2) Dimorphism: *The existence of two specific and distinct forms in one species, for example, male and female individuals.*

(3) Parthenogenesis: *Development of an ovum in the absence of a spermatozoon. 'Natural' parthenogenesis is found in many invertebrates (insects, crustacea), in which it forms a normal mode of reproduction. In some cases it appears to be accidental. In other groups, particularly in vertebrates, it only occurs as the result of experimental interference (amphibia and mammals).*

(4) *See the chapters on the paramecium (p. 103) and the freshwater hydra (p. 107).*

between males and females is nearly perfect, which suggests that the mechanisms responsible for this balance have become remarkably efficient. However, a certain lability[6] persists and we shall see that sexual organisms are fundamentally bipotential, and are in fact at the mercy of various influences which guide their development in the direction of one sex or the other.

We know that a strict causal relationship exists between the characteristic chromosome make-up[7] of a particular species and the sex of its individual members. The sex chromosomes, or heterochromosomes,[8] which can often be seen very clearly in the germ cells of one or the other sex, are mainly responsible for the sexual orientation assumed by an organism, which may be regarded as fundamentally bivalent. Yet, while the heterochromosomes play an essential role in the final expression of sex, the autosomes also exert some influence of a quantitative nature, for it has been found that the variations observed in the ratio between heterochromosomes and autosomes[9] or 'genetic balance', may sometimes determine the final sex of an organism.

It is also known that the sex hormones produced by the gonads act on the various somatic sexual characters in a selective fashion, and the very considerable mass of data which has been accumulated on this subject, has enabled us to use these substances therapeutically whether of a natural or chemical origin.

However, it must also be noted that the effectiveness of hormonal treatment may go well beyond a limited action on somatic characters. By experiments with grafting gonads or by prolonged courses of injections, it has in several cases been possible completely to reverse the sex of an animal. Thus in some amphibians (*Ambylostoma*) genetic females have been masculinised to the extent of acquiring all the characteristics of the male sex. These experimental males, have been shown to be capable of mating with and fertilising normal females. Conversely, feminised male toads have laid fertile eggs.

Thus, it is clear that the sexual balance of an organism is controlled by strictly specific internal factors. A conflict between opposing factors can on occasion bring about marked sexual anomalies, which often manifest themselves as states of intersexuality possessing varying degrees of stability. This problem has been studied in detail both by geneticists and endocrinologists. However we will now leave this to return to an earlier question: the action of external factors on sex determination.

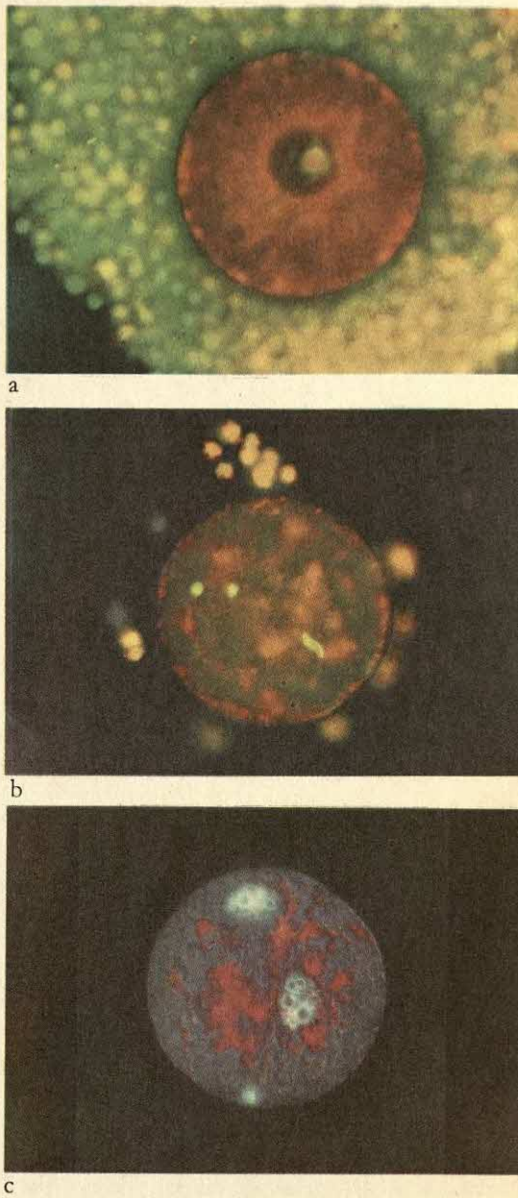

Fig. 4. Living ova of the rat at a very early stage of their development, seen under the fluorescence microscope (mag. × 500).
(a) The ovum before fertilisation.
(b) Fertilisation—the head of the sperm, which has just penetrated into the ovum, is visible at the top left as a green fluorescent ovoid. To the right and above there are the two masses of chromatin belonging to the ovum (one of the two will give rise to the polar body, to be eliminated later).
(c) Two cell stage. The green fluorescent bodies are the two nuclei, and, at the bottom, the polar body is being eliminated (photos, Autin and Amoroso).

Among higher organisms their effect is limited, but lower organisms are more dependent on extrinsic factors to control their sexual state.

Evidence in favour of sexual lability, that is the potentiality of an animal to develop into either a male or a female, is provided by the numerous cases of natural hermaphroditism which occur in lower forms. In these the gonads, and other attributes of the two sexes, may coexist or even be intermixed, or succeed one another at different times as distinct sexual phases. For example, the young animal may be a functional male but later in life become a female with female functions.

Numerous examples of such successive hermaphroditism are known among the molluscs. For example, *Crepidula*, a small marine gastropod lives in large populations within which groups of individuals of different sizes can be distinguished. The smallest are the males and the largest are females, while the medium-sized individuals are in the process of undergoing sex reversal (fig. 6). It can be shown that when very young individuals, still sexually immature, are brought up in isolation they change directly into females without passing through a male phase. By contrast, a young *Crepidula* which grows up in contact with a mature female becomes rapidly masculinised. Thus external stimuli influence the development of sex, but their exact nature has not been determined.

Another case which has been studied in detail is that of the marine annelid *Ophryotrocha*. The young animal is always male and does not become female until it has fifteen to twenty segments. As growth continues and the number of segments increases, the sex is to some extent stabilised. However, it is always possible to bring about secondary masculinisation of these females by surgical operation.
It is only necessary to starve them, or better still to cut them up in such a way that the anterior part with say only a dozen segments is isolated. This stump lives, produces male

(5) Sex-ratio: *The numerical distribution of the sexes in a species; examples: number of males to 100 females: cat, 107; mouse, 105; man, 104; sheep, 98.*

(6) Sexual lability: *The constitutional character which allows an individual in the course of development to become either a male or a female.*

(7) Chromosomal make-up. *The number and shape of the chromosomes in the nucleus which is characteristic for a given species.*

(8) Heterochromosomes: *Those which determine sexual differentiation: the sex chromosomes.*

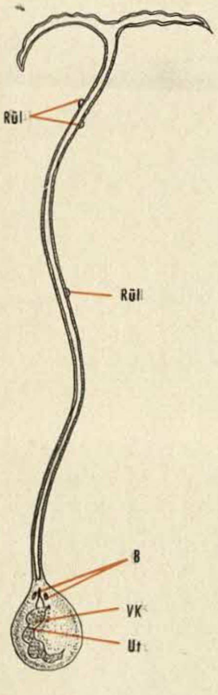

Fig. 5. Bonellia viridis. *An adult female. (Ut) the uterus containing the males (Vk). (Rül), larvae implanted on the trunk. B, bristles.*

gametes, and then begins to regenerate, so that when it reaches the 'female size' it undergoes sex reversal again. If, however, segments are repeatedly amputated the male state may be permanently maintained.

It has also been found that if two females are isolated one of the two individuals frequently becomes masculinised. The female whose sex is already most strongly determined starves her companion, or, more drastically, cuts her in half, thus rapidly providing herself with the male she lacked.

We will finally consider a third example of sexual bipotentiality, where change is possible as the result of the action of external factors. This is the sex determination in Bonellia, which was worked out by Baltzer. This marine worm is strange in many respects and belongs to the group of *Echiuride.* The female has an oval body equipped with a sort of extensible proboscis which can be extended to an overall length of about twenty centimetres. The male, which was for a long time unknown, was eventually discovered living in the oviduct of the female. It is very small and has regressed to a primitive state of organisation, assuming the habits of a parasite at the expense of the female. Thus one female may shelter up to eighty males (fig. 5). At this juncture it may be noted that the occurrence of rudimentary males is fairly common in the animal world, both among the invertebrates (for example, some Crustaceans) and the vertebrates (certain fishes).

But the real originality of *Bonellia* does not lie in this disparity in size, which is only an extreme exaggeration of sexual dimorphism, but in the factors which bring about the development of the male or female. At hatching, all the larvae are the same, whatever sex they will ultimately assume. Brought up singly in sea-water, they metamorphose progressively into typical females, but the picture changes if an adult female should happen to be present in the tank where the larvae are reared. It is then found that the larvae, still sexually undifferentiated, attach themselves to the female proboscis, presumably as the result of chemotaxis[10] that is, they are attracted from a distance by the diffusion of a specific chemical substance. Shortly after this fixation has occurred, that is, about four days later, almost all the larvae will have become males and soon afterwards they will migrate to the genital duct of the female. This masculinisation, which is in reality an inhibition of the female potentialities, may even be obtained by the action of an 'extract' of the female proboscis on the larvae. There is no doubt that the sex-determining agent is biochemical in nature, or that a genetic mechanism of limited effectiveness is present; for the few larvae which resist the action of the proboscis factor are irreversibly and genetically determined females.

This sexual lability is not the exclusive prerogative of the invertebrates but is also manifested to some extent by the vertebrates. A particularly clear example is to be found among the amphibians. Numerous geographic races of sexually 'undifferentiated' frogs have been found in various localities in Switzerland, Germany and France. The young tadpoles all appear to be females at metamorphosis, but the balance of sexes is re-established during growth, since about half of the apparent females become males by the time they are adult. The 'differentiated' races of these same frogs show a normal sex-ratio at metamorphosis, but if they are reared at a temperature lowered to 10°C, there is found to be a considerable excess of females.

The common feature underlying the few points that we have just related concerning the problem of sexual differentiation is that the final expression of sex apparently results from a conflict between various determining factors, which may be more or less prolonged. Thus, an individual is basically capable of developing into either sex, so that the accidental or experimental predominance of one factor over the others, sometimes leads to a completely aberrant result. All the various states of intersexuality, in fact, bear witness to the sharpness of the struggle for sex.

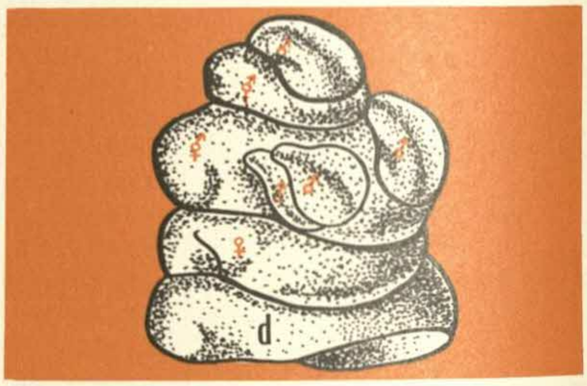

Fig. 6. Successive hermophroditism in Crepidula fornicata, *a gastropod mollusc. A group of individuals, some male (♂), some female (♀), and some in the course of sexual inversion (⚥), all fixed on an empty shell (d). The female at the bottom has been fertilised by the individual now in the course of sex-reversal, when it was in the male stage. The male at the top is ready to fertilise the one now in course of sex-reversal when it reaches the female stage. (After Coe).*

(9) Autosomes: *Chromosomes which are found in both sexes.*

(10) Chemiotactic attraction. *Attraction from a distance caused by the diffusion of a specific chemical substance.*

Problems of heredity

Pierre JOLY

As we have just seen, the egg and sperm, after they have met and fused, give rise to an organism which resembles its parents more closely than any others of the same species, and is more like the latter than any other living things. What is the basis of this remarkable process that reproduces the shape, appearance and behaviour peculiar to each species and each individual in every generation?

No two truly identical animals exist, except perhaps for identical (uniovular) twins.[1] However, not all individual differences are hereditary: some characters are only the response of the organism to environment and are not passed on to its descendants.

For example, the migratory locust has a green colour when it lives in a scattered population, but, when it lives in a very dense population, it has a brown and black pigmentation (fig. 1). If the descendants of a couple of brown animals are isolated, these may assume the green pigmentation of solitary animals, and *vice versa*, if the descendants of green animals are brought together, they can take on the brown colour of animals living in dense populations.

In contrast to this, hereditary characters are transmitted to the offspring in spite of all the possible fluctuations in environmental conditions, and continue to be passed on for many, if not an unlimited number, of generations. One example is albinism among mammals: provided a strain of white mice is not crossed with any grey mice, it will give rise only to white ones, under any conditions.

Different individuals of the same species must possess an enormous number of hereditary characteristics in common. In fact, the concept of a species depends on this: such characteristics are necessarily hereditary.

Since the beginning of the twentieth century, the study of the transmission of hereditary characteristics has been the subject of a series of important studies. Among them are those of Morgan and his followers on the fruit fly, *Drosophila melanogaster,* which are of major importance, and all this work indicates that the significant part in these mechanisms is played by the cell nucleus and its chromosomes. However, as we shall see later, there is another and quite different type of inheritance known as cytoplasmic heredity.

Nuclear heredity

Once it is accepted that the genes are carried on the chromosomes, it becomes possible to explain and even predict the laws of heredity. During an organism's growth, the nucleus multiplies by mitosis.[2] This process (the principal phases of which are recalled in fig. 2) provides each cell with a chromosome set exactly identical to that of the cell which gave rise to it. The genetic material, or genotype, is transmitted equally to the daughter cells.

When the reproductive cells or gametes are formed, a special nuclear division, known as meiosis, takes place (fig. 3). In the course of this division, the chromosomes of a like pair come close alongside each other (synapsis) and, following this, they separate and each goes to one of the poles of the division. Thus, meiosis, instead of separating the two halves of each chromosome, separates the two chromosomes of a like pair, so that each gamete contains a single representative of each chromosome pair. At fertilisation, the original number of chromosomes in the egg is re-established by the fusion of the egg and sperm. This mechanism has the following consequences: each fertilized egg, each new organism, possesses the same number of chromosome pairs as its ancestors, and, in each of these chromosome pairs, one chromosome comes from its mother and the other from its father. Thus, one of the two homologous genes,[4] that is genes which are responsible for the same characteristic such as eye-colour, comes from its mother and the other from its father.

Conversely, among either male or female progeny, one or other of the representatives of each of the chromosome pairs has an equal chance of being passed on to its immediate descendants, whether these are male or female. Consequently the same must apply to either

---

(1) Uniovular twins: *two sorts of twins occur in man and other mammals. First, binovular twins, which arise from two separate ova produced and fertilized at the same time. They are no more closely related than other brothers and sisters. Second, uniovular or identical twins, which arise from a single egg which has divided into two embryos in the course of development, so that they are really two parts of the same organism.*

(2) *The various phases of mitosis are described in detail in the chapter 'From cell to tissues', page 59.*

(3) *Among animals, one of the sexes has two chromosomes which are not a pair. In most cases, particularly in man, the two unpaired chromosomes occur in the male and are known as the* X *and* Y *chromosomes. In the female, there is no* Y *chromosome, but a pair of chromosomes.*

(4) Alleles: *a gene may have two or more different forms. One of these corresponds to the normal gene, the others to the mutant genes, which are called alleles of the normal gene.*

(5) Usual genetic notation: *The first generation used in an experiment, or parental generation, is called* P. *The first, second and third generation progeny are called* F1, F2, F3, *etc. All the abnormal genes are given a letter (or group of letters): a small letter if it is recessive with respect to the*

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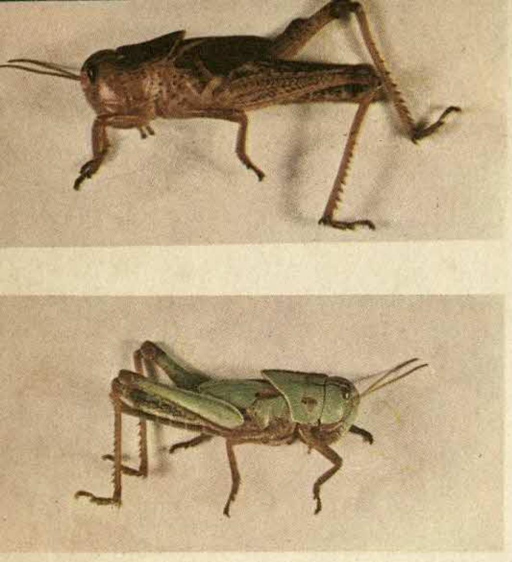


Fig. 1. (a) Larva of Locusta migratoria *from a dense population. (b) individual reared in isolation (Photo P. Joly).*

Continued from previous page

corresponding normal gene, and a capital letter if it is dominant. The normal gene is also called the wild gene and is designated by the same letter together with the sign $^+$. *Thus, in* Drosophila, *the gene controlling the character vermilion, which is recessive, is called v and the corresponding normal gene, which gives the wild colour of the eye,* v^+. *The gene Bar, which is dominant with respect to its wild allele, is called* B. *The genetic make-up or genotype of an individual is given in the form of a fraction in which the two terms represent the chromosomes of a homologous pair: a 'vermilion' fly has a genotype* v/v. *If it is crossed with a wild fly* v^+/v^+, *the heterozygote* v^+/v *is obtained. The appearance of the animal, or its phenotype, is represented by a letter in brackets. The fly* v/v *has the phenotype* (v), *compared with the flies* v^+/v *which have the phenotype* (v^+) *because of the dominance of* v^+.

(6) Giant chromosomes: *The growth of some organs in the larvae of various insects takes place without cellular multiplication: the embryo's cells only increase in size. Thus some cells become giant cells and have nuclei with giant chromosomes. During growth the chromosomes multiply just as if cell divisions had taken place, but, instead of separating, they remain together side by side. (This phenomenon is called endomitosis.)*

of the two genes which determine each of its characters (except for those carried by the X and Y, the sex chromosomes).[3]

This brings us to the empirical laws of Mendelian heredity.[5] For example, if a normal grey mouse is crossed with a white mouse (albino) and we consider only the chromosome pair carrying the genes controlling pigmentation, then we know that each of the offspring receives one chromosome from its normal parent and one from its albino parent. Consequently it has one normal gene *E* and an albino gene *e* (fig. 5). This first generation offspring is grey, demonstrating the phenomenon of dominance, which is very common but not universal: the F1 generation may be intermediate between the two parents.

If the mice of the F1 generation are now crossed among each other, the grey and white characters are seen to segregate out again (fig. 6). Each of the F1 generation progeny provides as many gametes with an *E* gene (grey), called the dominant gene, as gametes with a chromosome carrying an *e* gene (white), called the recessive gene. Since, at fertilisation, the eggs and sperms meet at random, the four possible combinations *E/E*, *E/e*, *e/E* and *e/e* have equal chances of being produced. Therefore, if the F2 generation is sufficiently numerous for the laws relating to large numbers to apply, there will be 25 per cent grey individuals identical to the grey individuals of the P generation, 50 per cent grey individuals identical to the grey individuals of the F1 generation (taking into account that *E/e* is identical to *e/E*, for it is unimportant whether the gene comes from the father or the mother) and 25 per cent white individuals identical to those of the P generation. In all, taking dominance into account, we arrive at the well-known proportions: $\frac{3}{4}$ grey and $\frac{1}{4}$ white.

Chromosomal structure and the distribution of genes

If we examine ordinary histological preparations we may be led to think of the chromosomes as rods containing genes just as a long tube might contain balls. However, various methods of investigation have enabled us to examine this structure and the reality is very different. One method consists in examining the chromosomes at the end of prophase when they are already stainable, but before contraction and spiraling have given them the compact structure of metaphase chromosomes. Another method investigates organisms with large chromosomes such as the giant chromo-

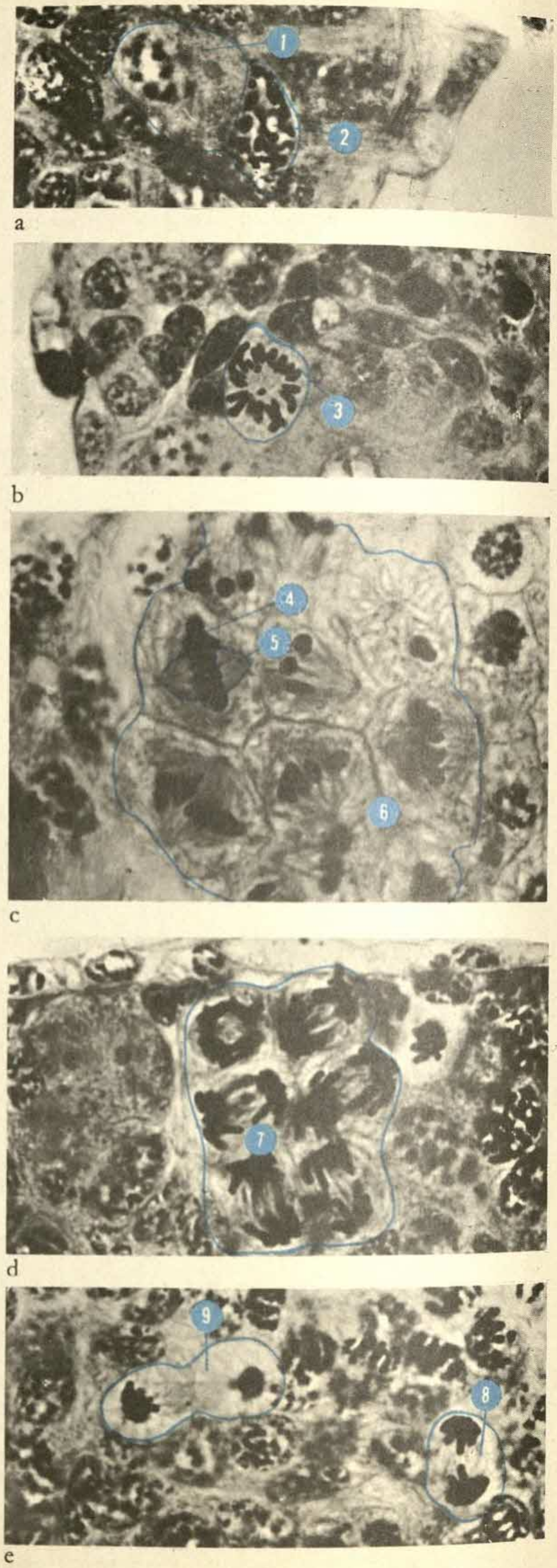


Fig. 2. Various phases of mitosis in Locusta migratoria, *the migratory locust. (a) Spermatocyte in interphase (1) and vegetative cell in prophase (2). (b) Polar view of metaphase (3). (c) Equatorial view of metaphase (6). The chromosomes on the equatorial plate (4) and the achromatic spindle (5) are clearly shown. (d) Anaphase (7). (e) End of anaphase (8) and telophase (9) (Photo Joly).*

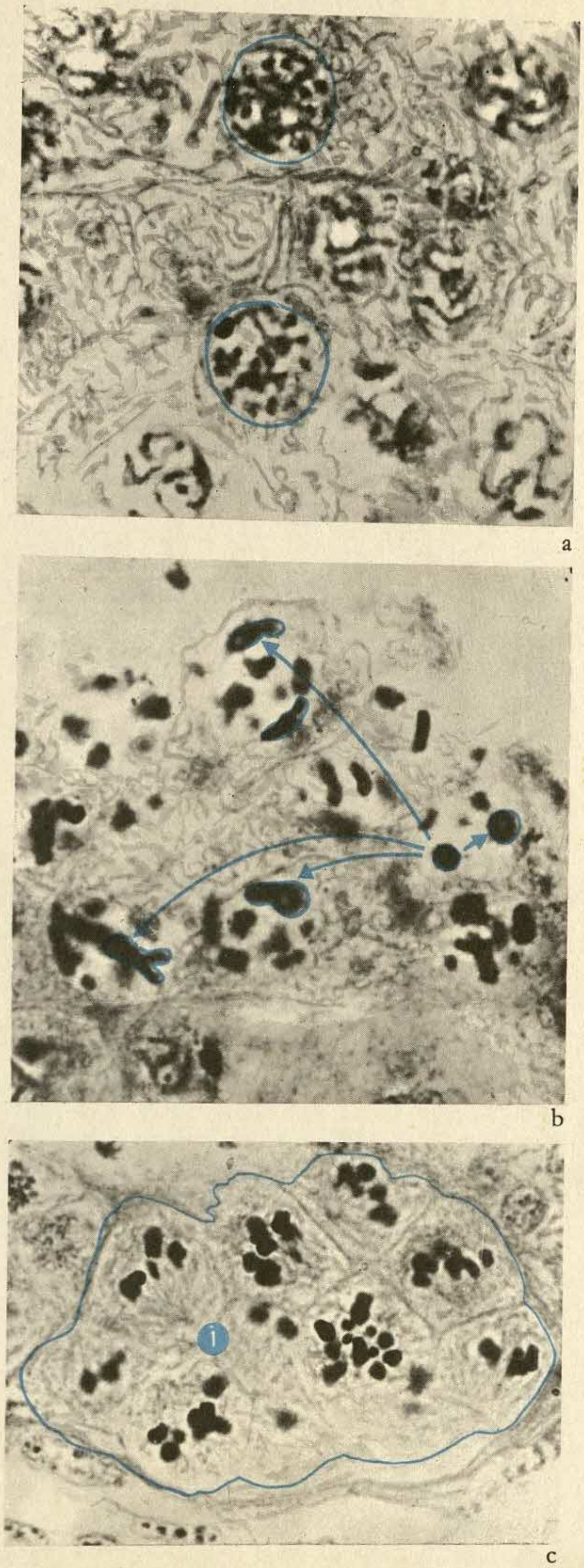
somes[6] of some Diptera larvae (fig. 4) which have provided much information in this field. More recently, complementary data has been provided by the electron microscope.

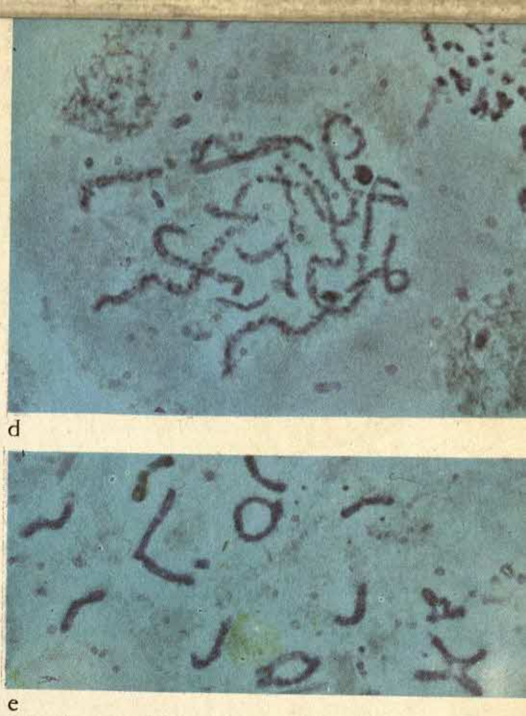
All these investigations lead us to regard the chromosomes as made up of a very fine filament called the *chromonema* bearing at intervals along its length small granules known as *chromomeres* (fig. 7a). The nature of the latter has been disputed by many authors who consider them to be small localised coils in the chromonema (fig. 7b). The chromonema also carries a single special granule, the centromere, by which the chromosome is attached to the achromatic spindle. Finally, in the metaphase chromosome, the chromonema is rolled up into a helix or spiral and covered by a sheath called the matrix (fig. 7c).

However, the structure of the chromosomes seems to vary according to both the organism investigated and the conditions under which it is functioning. By means of an electron microscope, Grassé has shown that the chromosomes of snail spermatids[7] during interphase are made up of chromonemas in the form of long, perfectly homogeneous filaments of 230 to 240μ in length and 70–100 A in diameter. These are folded on themselves many times, but show no trace of spiraling or chromomeres: the matrix is no longer visible.

The very peculiar structure of the giant chromosomes of the Diptera can be seen under the ordinary microscope. They are some several hundred μ long (485μ for the whole of the two arms of chromosome III of *Drosophila melanogaster*) and have a diameter of several microns. They are made up of a bundle of chromonemas which are not spiralled and very numerous (1,600 in *Drosophila*). This gives the length and diameter of the giant chromosomes. Furthermore, the two chromosomes of a like pair are close together side by side and rolled round each other. The whole appears to be transversely striated and, in fact, these bands or transverse discs, are made up by the juxtaposition of the homologous chromomeres of the various chromonemas making up the two chromosomes. The bands are very varied in appearance, so that accurate topographic marking can be carried out with this kind of chromosome. The structure of chromonemas constituting the chromosomes of higher organisms has been compared to that of rod-like viruses or the central body of bacteriophages.[11]

The filamentous structure of the chromosomes explains how they function. We have seen that they carry the material of the genes, but, since the number of pairs of chromosomes





Figs. 3. a, b and c; prophase of meiosis in the migratory locust. (a) After interphase the chromosomes appear in the nucleus and shorten and thicken (Pachytene). (b) Pairing and the beginning of dislocation of homologous chromosomes (Diplotene). (c) The chromosomes of each pair separate (Diakinesis), in such a way that each cell receives half of the parental set of chromosomes. (d) Pachytene, (e) Diplotene stages (photos Pierre Joly).

(7) Spermatids: *the sex cells have a long and complicated development. First, large clear cells, called primary gonocytes differentiate in the gonads of a young embryo. In the male these give rise to smaller cells, the spermatogonia, which multiply and then grow, giving rise to spermatocytes* I. *Each spermatocyte undergoes meiosis, which reduces its chromosome number by half, and produces two spermatocytes* II. *Each of these then divides into two spermatids. Each spermatid then changes into a spermatozoon.*

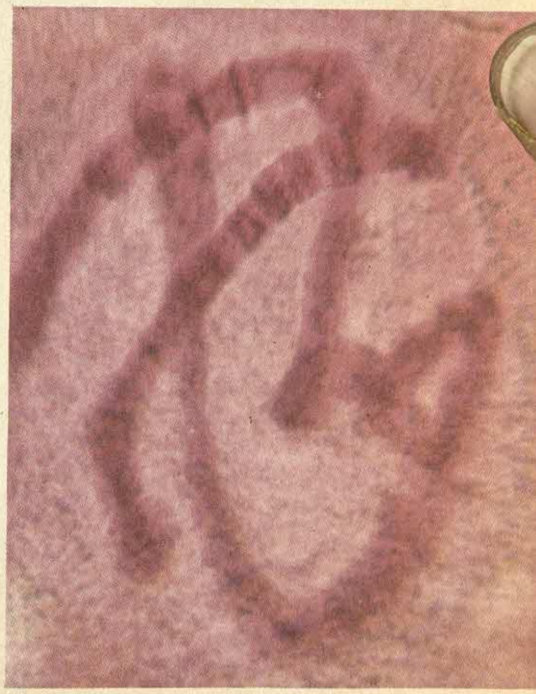


Fig. 4. Giant chromosome of Chironomus *larva (photo Lod).*

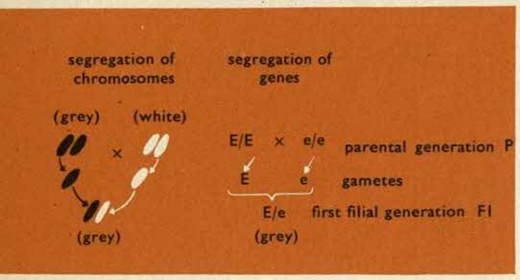


Fig. 5. Distribution of a chromosome pair in a cross between two mice of pure strain, one grey and the other white.
P, *parental generation.*
F1 *first generation offspring.*

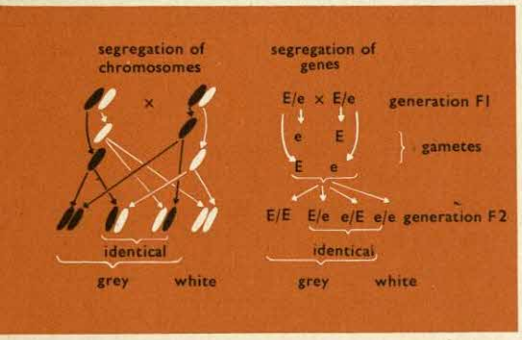


Fig. 6. Distribution of a chromosome pair in a cross between two hybrid individuals.
F1, *hybrid individual of first generation.*
F2, *second generation.*

in an organism is much less than the number of genes, each chromosome must carry an enormous number of genes. A fairly short chromosome carries many hundreds of genes and it is tempting to locate them on the chromomeres.

Since the different genes of the same chromosome have been carried by the same bearer, we would expect them to be inherited together as one group. This certainly happens in most cases when the genes and the characters that they control are said to belong to the same linkage group. For example a 'cinnabar' drosophila with bright red eyes (*cn*) and black body (*b*) may be crossed with a wild drosophila with red-brown eyes (cn^+) and grey body (b^+). The genes *cn* and *b* are both located on the second pair of chromosomes and one might therefore expect to get only drosophila with bright red eyes and black bodies or those with red-brown eyes and grey bodies. But, among the second generation several individuals are found which combine the character of bright red eyes with a grey body and others which combine red-brown eyes with a black body (figs. 10 and 8).

It has been shown that the following events can take place at meiosis: the two chromosomes of a like pair, when brought together in synapsis, may break and rejoin at any point and thus be exchanged along part of their length. This is called crossing-over. If this takes place in a heterozygote[8] of the F1 generation and the break occurs between the points where the genes cn and b are located, it results in the gametes recombining cn with b^+ and cn^+ with *b*. These are the gametes responsible for the individuals recombining the characters of their grandparents.

If one assumes that the probability of a chromosome pair breaking during meiosis is independent of the genes they carry and that the break may occur at absolutely any point (which is true at least at first approximation) it is clear that the break has more chance of falling between two given genes, and thus resulting in recombination, the further apart they are on the chromosome. Thus, by recording the frequency of second generation individuals which have recombined their grandparents' characters, we have a method for evaluating the distance apart on the chromosomes of the two genes which control these characters. If this process is carried out step by step for all the genes carried on a like pair of chromosomes, that is, all the genes belonging to the same linkage group, we can draw up a genetic map of chromosomes.

Laws of chromosomal heredity

Thus chromosomal heredity brings us back to two fundamental phenomena. First, Mendelian segregation, which is the consequence of chromosome segregation, and second, recombination, or crossing-over, which is the consequence of the exchanges between homologous chromosomes. These phenomena have been found in all organisms, ranging from the micro-organisms (bacteria and viruses) to man, and including cultivated plants (maize, corn, tomato), domestic and laboratory animals (drosophila, mice, etc).

Obviously it is impossible when studying human genetics to arrange matings as we please. However, the available populations are so plentiful, that by looking around carefully enough, almost all kinds of union can be observed. Besides, whilst it is impossible to follow a genealogy for many generations, and the numbers in the families of each generation are always small, these difficulties may be overcome by considering a large number of family genealogies at the same time, in which the character whose transmission is being investigated is segregated.

Various methods have been devised to solve this type of problem. The principle underlying them is nearly always the same: a hypothesis is made as to the mode of transmission of the character and it is confirmed whether or not the theory is in agreement with the observations.

Take, for example, the case of albinism. It is known that, in animals, this character is controlled by a recessive gene in the homozygous state e/e. So, in man, the following questions may be asked: is albinism recessive and are the Mendelian proportions of a monohybrid observed in the families where this character is segregating? If so, the following facts should be noted:

First, in those families where both the father and mother are albino, only albino children should be produced: *e/e* × *e/e* gives *e/e*. This case is very rare.

Second, in families where one of the parents is albino, two situations are possible:

(a) The second parent does not have a gene for albinism and all the children should be normal. This is the commonest situation: *e/e* × *E/E* gives *e/E*.

(b) The second parent is heterozygous for the gene for albinism. Then half the children should be albino: *e/e* × E/*e* gives $\frac{1}{2}$ *e/e* + $\frac{1}{2}$ *E/e*.

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(8) Heterozygote: *an individual in which the two genes of a pair are represented by different alleles, for example vermilion and wild* v/v^+ *(drosophila eyes).*

(9) Monohybridism: *the name given to the type of segregation resulting when two individuals differing by a single character are crossed, eg a grey mouse and a white mouse. If the first type is dominant over the second, such a cross gives proportions of* $\frac{3}{4}$ *of one type to* $\frac{1}{4}$ *of the other in the second generation.*

(10) *It may be predicted that in such a family of four children, there is approximately one chance in three that all will be normal. That is, each infant has three out of four chances of being normal at birth. Thus the probability of a normal child being produced four times in succession is* $(3/4)^4 = 1/3.16$.

Third, the two parents are normal, but there are some albino children. This is because the two parents are heterozygous for the gene for albinism and one quarter of the children will be albino: E/e × E/*e* gives $\frac{1}{4}$ *e/e* + $\frac{1}{2}$ E/*e* + $\frac{1}{4}$ *E/E*.

However, before comparing these deductions with data provided by the study of a human population, it must be noted that a family of the type 2b, which has only a small number of children, is very likely not to have any albino ones and it will then count as 2a. In the same way, a family of type 3 may very well have no albino children and thus completely escape the enquiry. Thus, if we count all the children of type 3 families which can possibly be detected, the proportion of albino children will be very high. But this is a problem of a purely mathematical nature which can be solved without difficulty in a perfectly straightforward way. It is, in fact, fairly easy to show that the true proportion of albino and non-albino children among the offspring of heterozygous parents is discoverable if we exclude from the count that child whose presence has led to the detection of the family. The same result is obtained by subtracting one from the total number of albino children in all those families where parents of normal appearance have produced at least one albino child. It is thus possible to compare the hypothesis with the facts.[10]

It is then found that two types of albinism occur in man. One is recessive, fairly common, and strictly in accordance with the laws of monohybrid[9] segregation. The other is a rarer albinism determined by a dominant gene.

The problem of albinism which we have just considered is one of the simplest situations in human genetics. However, in many cases the mathematical analysis of data gained from the observation of populations is rather laborious and where the required methods did not already exist, mathematicians have had to develop them.

Such investigations have enabled human genetics to make rapid progress. Today, the mode of transmission of a very large number of genetic characters in man such as hereditary diseases, antigenic characters, biochemical characters, racial characters, etc., has been accurately determined. These studies show that the laws of Mendelian segregation and crossing-over apply in man as they do in animals. Even the study of linkage groups has been undertaken and the genetic map of certain chromosomes sketched, particularly that of the X and Y chromosomes (fig. 13). Nevertheless, most of these investigations are still at a very early stage.

At the other end of the scale of living things, the genetics of micro-organisms has made several years of spectacular progress, and it has been possible, by making some slight amendments, to apply the fundamental laws of genetics to these and similar forms.

The phenomena of sex in bacteria have been known in some detail since the work of Lederberg and Tatum. For example, in *Escherichia coli* conjugation is found to take place between + and − bacteria in a way which is comparable to true fertilisation. The + bacterium plays the male role and transfers its genetic material to the − bacterium playing the female role.

If the two bacteria taking part in conjugation come from two different strains (differing, for example, in their capacity to live on a culture medium deprived of biotin, cystine or leucine, or by their resistance to penicillin or some strain of bacteriophage), then the descendants resulting from this cross show segregation and recombination of parental characters which may be explained as follows: the bacteria under consideration are haploid[12] and each has a single chromosome carrying the genes arranged in linear order. During conjugation, only a part of the + bacterial chromosome (donor) is transmitted to the partner, leading to a sort of incomplete zygote. In this zygote a phenomenon that is closely analagous to crossing-over takes place between the two chromosome elements, resulting in the recombination of certain parental characters as in higher organisms. This zygote[13] stage is often very brief and, after the following division, the haploid state is resumed.

Genetic processes in viruses are even stranger, but the basic facts are the same. The 'genetic information' is borne by a linear structure carrying a set of genes in fixed sequence.

Bacteriophages, for example, have a central body composed of deoxyribonucleic acid, which is comparable to a chromosome. Only this body penetrates into a parasitised bacterium, where it can, in some cases, be incorporated into the genome[14] at a clearly defined locus[15] and multiplies along with it. If a bacterium is infected simultaneously with two bacteriophages differing by a certain number of characters, these characters are found to be capable of recombining and segregating out in the phages which come from the bacterium. The study of these phenomena enables us not only to show that the phage characters are

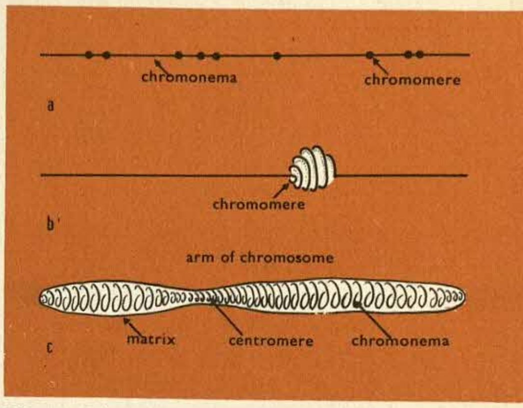


Fig. 7. Diagram of the structure of a chromosome.
(a) Chromonema with chromomeres.
(b) Hypothetical structure of a chromomere.
(c) Structure of a metaphase chromosome.

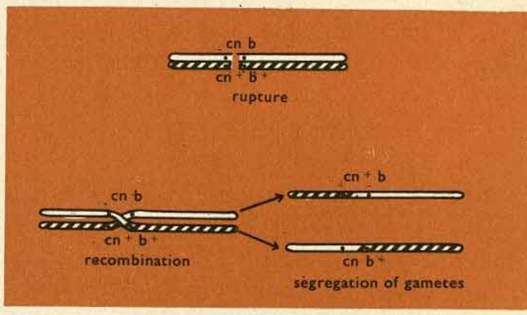


Fig. 8. The recombination of genes cn and b^+ *in a heterozygote by cross-over, the breaking and exchange of the ends of the chromosomes.*

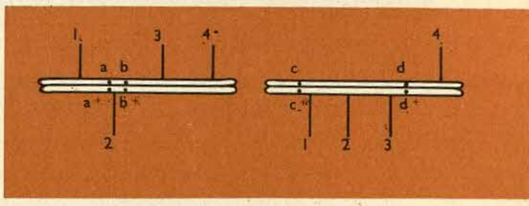


Fig. 9. On the left, two genes a and b^+ *which are situated close together, are only able to recombine if the cross-over takes place at level 2. A break in the regions 1, 3, 4, does not enable them to recombine. On the right, two genes situated at c and* d^+ *may be recombined by breaks at 1 as well as 2 or 3. Only a break at 4 fails to bring about their recombination.*

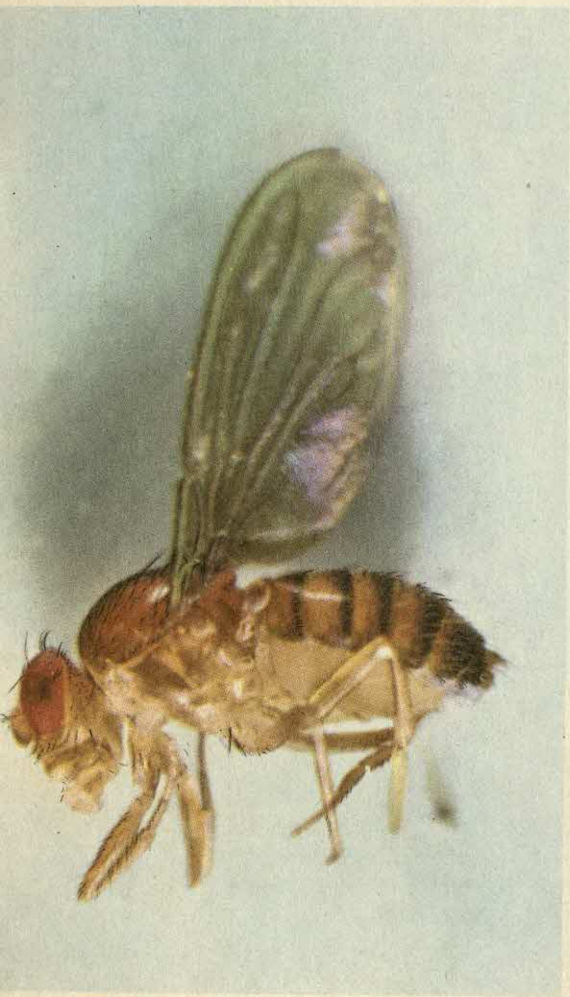
Drosophila mutations

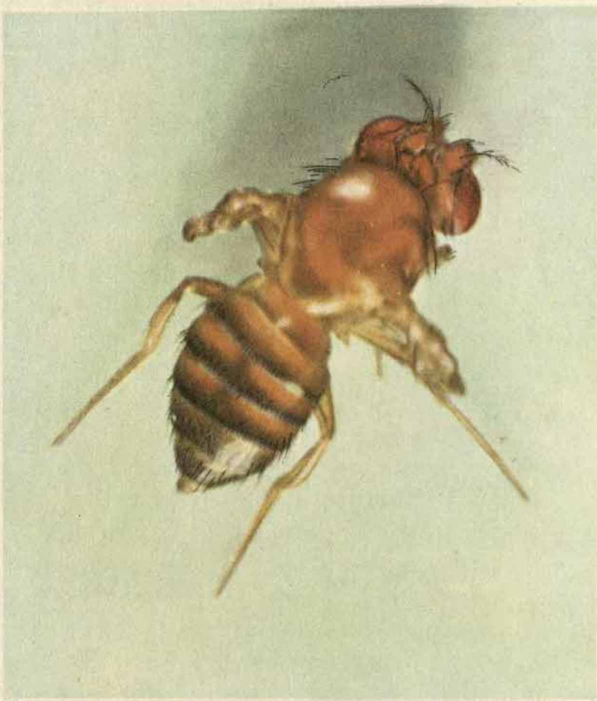
Drosophila melanogaster *(fruit fly) is a valuable organism for the geneticists on account of its rapid reproduction, its giant chromosomes and its spectacular mutations. Some of these mutations are shown here.*

(11) Bacteriophages *(abbreviated to phages): viruses which are parasitic on bacteria. The virulent forms kill the bacterial cells, which are described as being lysed. The non-virulent, or temperate phages, can reproduce symbiotically within the bacteria for many generations without producing lysis.*

(12) Haploid: *an organism in which the chromosomes are single and not made up pairs as they are in diploid organisms.*

fig. 10. - A





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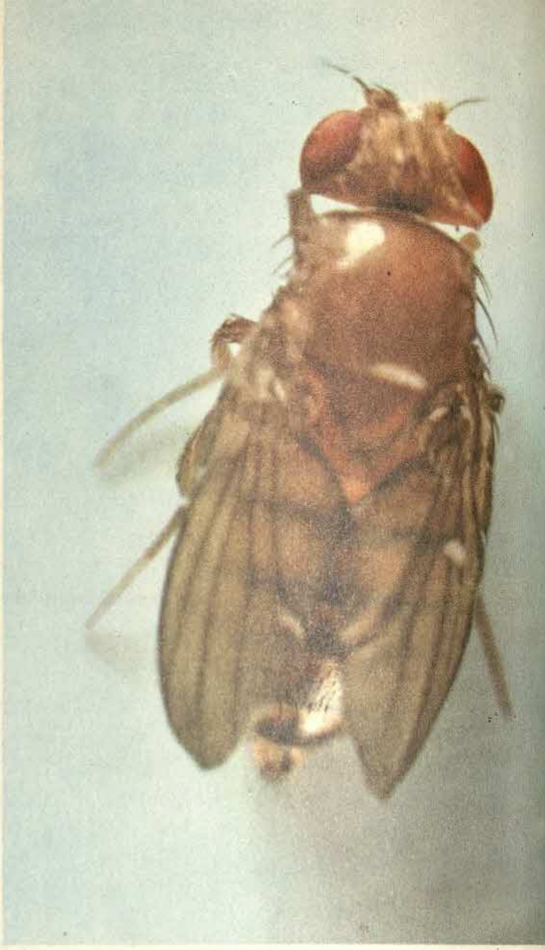

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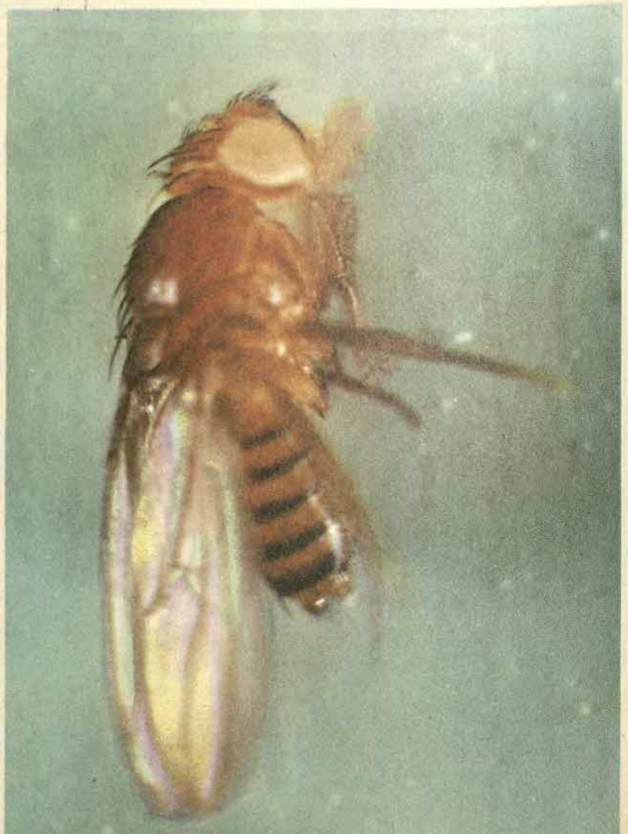
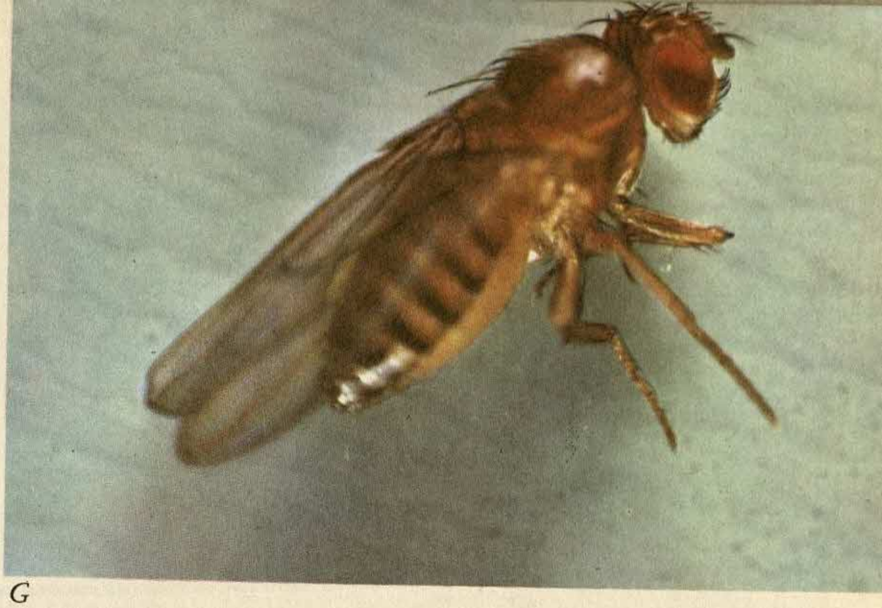


These photographs appear by courtesy of M. Bösiger of the C.N.R.S.



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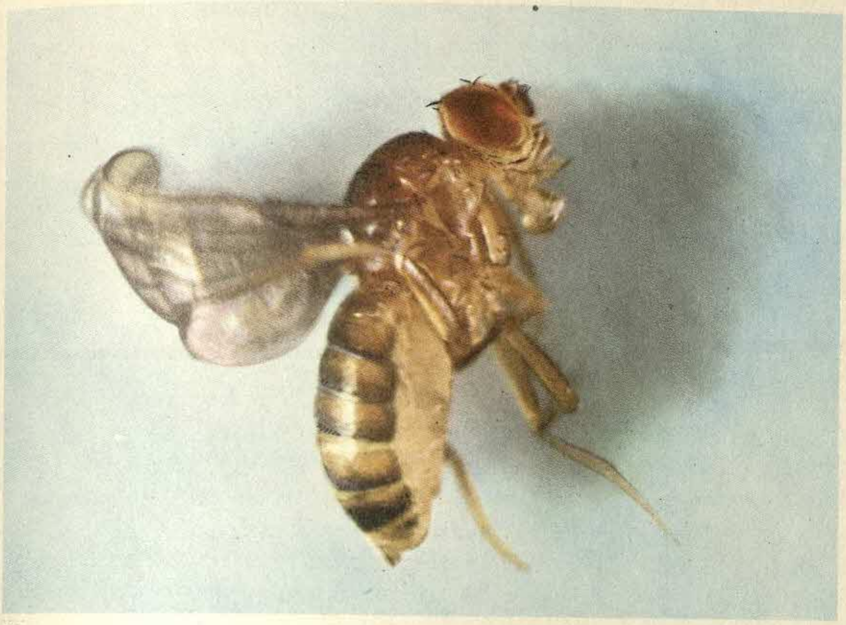
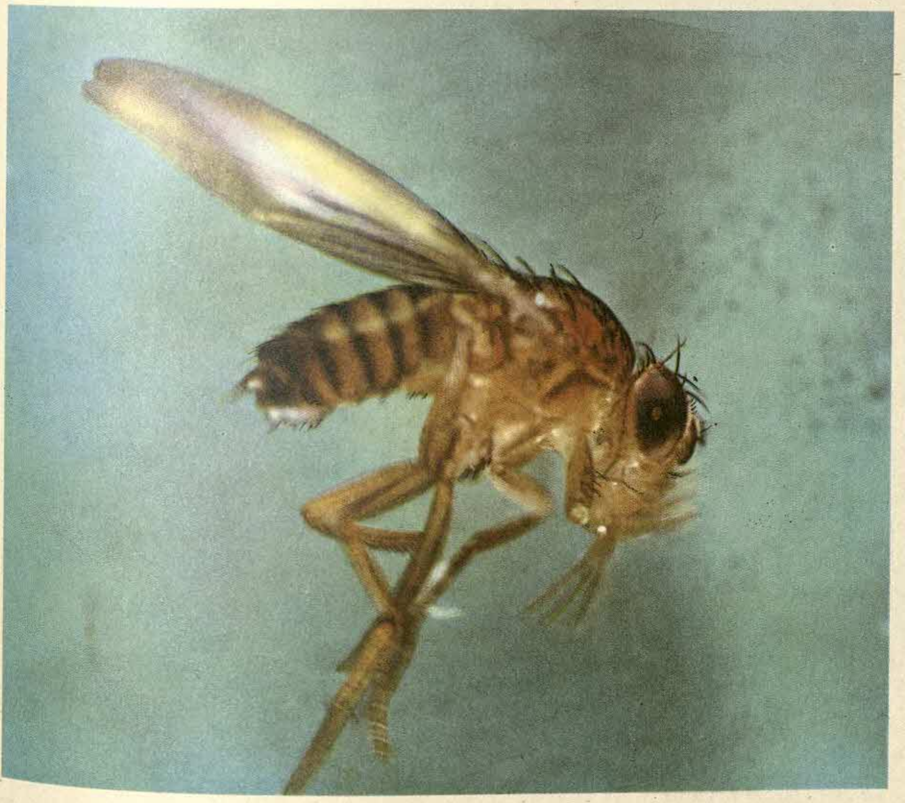


E

Fig. 10. Drosophila *Mutations*

A: female, brown eyes, veined wings.
B: female, vestigial wings.
C: female, bombee, short and less transparent wings.
D: female, white eyes.
E: female, curly wings, plum eyes, stubble bristles.
F: female, sepia eyes, dark body.
G: female, scute, wavy wings.
H: male, coral eyes.
I: male, dark body, maroon brown eyes (p. 124).
J: male, cinnabar eyes, wavy wings (p. 125).
The following are wild characters: the eyes of flies B *and* C; *the wings of flies* D *and* F; *body and bristles of flies* A, B, C, D, H *and* I. *All the other characters are results of mutations. The best-known are those of flies* B *(vestigial wings),* D *(white eyes),* E *(curly wings, plum eyes, stubble bristles),* F *(sepia eyes),* G *('scute'),* J *(cinnabar eyes). (Mag. × 50 to 100, Photos Lod.)*

F



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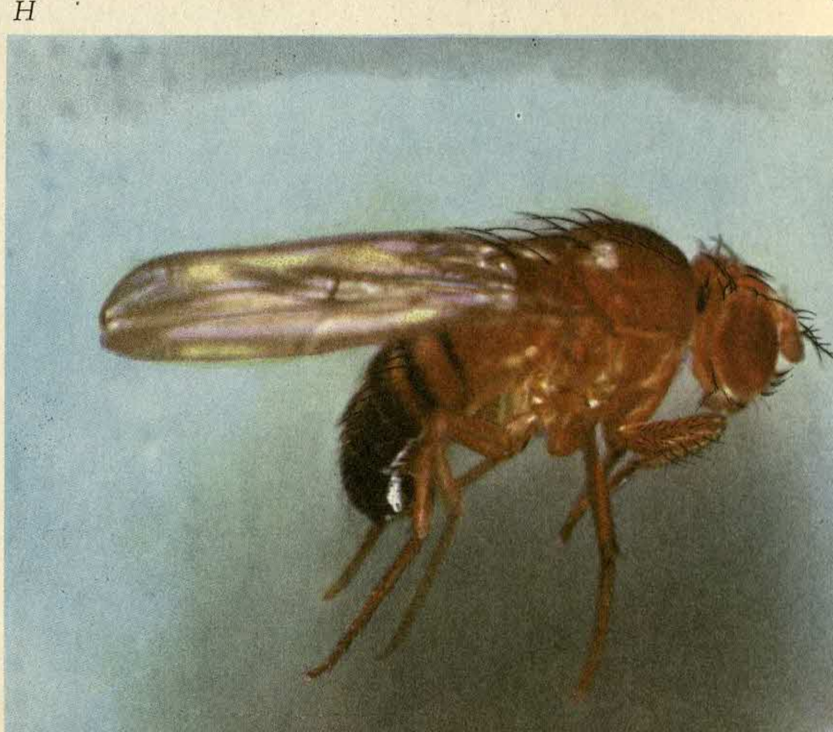

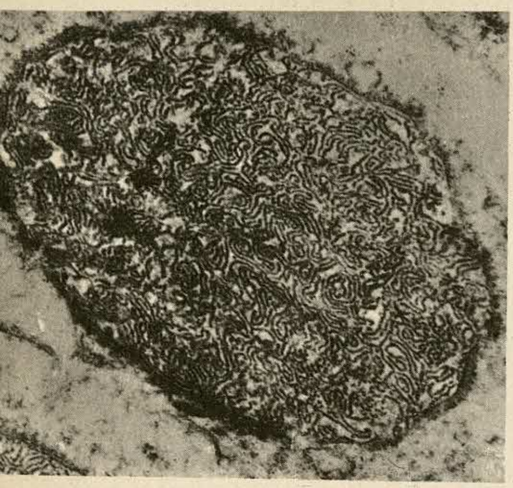

Fig. 11. Chromosomes of snail spermatids photographed under the electron microscope (Pierre P. Grassé).

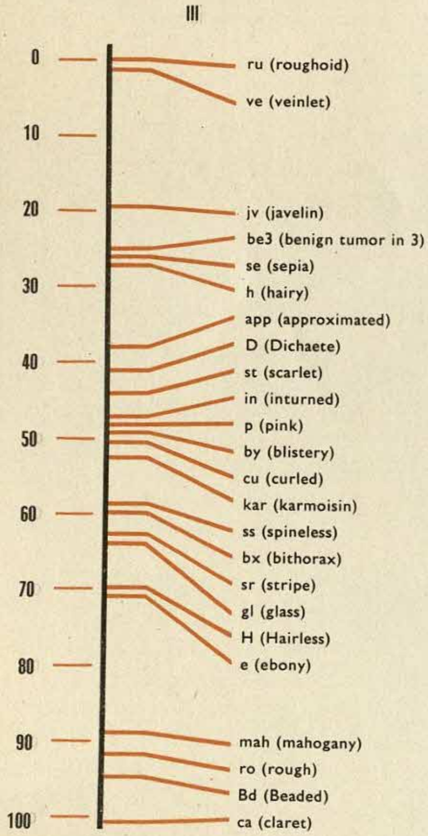

Fig. 12. Genetical map of the 3rd chromosome of Drosophila melanogaster. *Only some of the known genes are represented on this map. The capital letters indicate that the mutant gene is dominant over its wild allele.*

controlled by a linear sequence of genes, but even to draw up a detailed map of their positions along the supporting filament. Thus we have reached the paradoxical situation in which the structure of the chromosomes is best known in a virus.

Function and definition of genes

We have just considered the gene as a factor which controls the appearance of a definite hereditary character. For example, there is the gene for albinism, known in all mammals; the gene for alcaptonuria which occurs in man, resulting in the urinary excretion of homogentisic acid; the Bar gene, which transforms the eye of drosophila; and so on. However, the gene may also be defined by its ability to mutate. Thus in a completely pure strain of white *Drosophila* with yellow eyes, an individual with red eyes eventually appears. The gene controlling eye pigmentation has changed from the state w to the state w^+ (fig. 10).

It is this very fact, that a gene mutates, that enables us to know of its existence. For instance, an anomaly of the human skin, in which it is thick and scaly like the skin of a fish, has been described as *Ichthyosis histrix gravior.* An examination of the very long Lambert family geneology showed that this is controlled by a gene located on the Y chromosome. The Lambert family is the only known case and the gene *Ichthyosis histrix gravior* has appeared only once in 1717, in Edward Lambert. If this mutation had not appeared, we would not know that the normal structure of the human skin is controlled by a gene which, among others, is localised on the Y chromosome and is an allele of the gene for *Ichthyosis histrix gravior.*

Finally, a gene may be defined by its position on the chromosomes; in fact, most genes have multiple effects. In albino mammals, the skin is white and the eyes are red.[16] These characters may be due to distinct genes situated on the same chromosome and therefore often inherited together, or, alternatively, they may be due to a single mutant. In the first instance, the fact that the phenomenon of crossing over may take place anywhere along the chromosome, will always, at some time or other, end up by separating the two characters 'red eyes' and 'white hair' which will segregate in the offspring. In the second case they will never be separated.

This concept of the gene accounts completely for the genetic phenomena observed in current experiments and will certainly continue to remain valid for a long time as a first approximation, although it has sometimes been necessary to make various amendments to it in order to take into account the most recent advances, particularly those resulting from work on the genetics of micro-organisms.

Pseudo-allelism

The state of our knowledge today makes it no longer possible to regard the gene (in the classic sense of the term) as an indivisible unit, or 'genetic atom'. In fact, a large number of them have been shown to be made up of many smaller units, such as the genes for white, vermilion and forked, to cite only three well-known examples from *Drosophila* genetics. However, the separation of these subsidiary units is often extremely difficult. How, then, can we tell if two characters are controlled by one and the same gene, or by two genes situated at two distinct loci?

The normal method consists in trying to separate them by crossing-over. To do this, matings are carried out which will put the two loci, which are presumed to exist, into the heterozygous state. Then recombinant individuals are sought among the progeny of these heterozygous individuals. For example, if the respective parents show the characters A and B' in one and A' and B in the other, then the presence of some AB and A'B' individuals, that is, those individuals recombining the characters provided by the two ancestors, is looked for in the second generation. If such individuals exist, then the characters A and B are controlled by two distinct genes that have been separated in the recombinant descendants by crossing-over.

But we also know that the frequency of recombination following crossing-over is proportional to the distance apart on the chromosome of the two loci involved in the cross under consideration. If these loci are sufficiently near, then the frequency of recombination will be so small that there will be very little chance of recovering any without working with huge numbers, so that B' will appear to be the allele[4] of A'. These are known as 'pseudo-alleles'. In higher organisms, where it is impossible to work with sufficient numbers, these pseudo-alleles cannot be investigated as such, except by the use of more or less indirect methods. Matters are also made more difficult as the phenotypes of the two mutants A' and B' are more often than not alike, if not identical.

Among micro-organisms, research on pseudo-alleles is much easier, firstly, because millions of individuals may be raised in a culture tube and experiments can be carried out on such large numbers that even the most improbable genetic combinations will always be realised. Secondly, more often than not, the genetic characters studied in these organisms lend themselves to methods which make it possible for the various phenotypes to be sorted out automatically, thus greatly simplifying the search for recombinants. For example, Demerec, working on *Salmonella*, obtained eleven tryptophane strains (that is, strains requiring the presence of this substance in their culture media). In order to tell if two of these strains were identical, or alternatively came from two distinct mutations at more or less widely separated loci, it was sufficient to permit genetic exchange to take place between them and then to culture them on a medium which was lacking in tryptophane.

In the first case, the chromosome region is present in an altered state in both of the partners and there is no possible recombination which could provide an individual with a normal chromosome. By contrast, in the second case, the whole chromosome region is present in a normal condition in at least one of the two partners. Therefore it is possible in the course of the recombinations for at least some individuals to be produced whose chromosome carries neither of the two alterations and which, because of this, are able to grow on a medium without tryptophane. Thus Demerec has shown the existence of pseudo-alleles having the same phenotype.

Whatever organism has been investigated and whatever technical details have been used, where the method has been adequate, the loci of classical genetics have had to be divided up into smaller units. There is nothing remarkable in this fact itself, and it is natural to imagine, *a priori* that some loci will be so near to each other that it is very difficult to separate them. But what is remarkable, and justifies the idea of pseudo-alleles, is that those mutations, which are located at points sufficiently close to each other on the same chromosome, all have the same phenotypic effect: the mutations *Star* and *Asteroid* produce the same deformation in the eye of drosophila; the genes v^s and v^A (both corresponding to the old vermilion locus) produce the same red eye colour; and the tryptophane pseudo-alleles, all produce the character of being unable to grow without tryptophane, etc. The term cistron is used for the chromosome segment along which the different pseudo-allelic mutations producing the same phenotypic effect, are located.

Bacteriophage chromosomes

Among the organisms which can be analysed most readily, like the phages, a topographic study of the possible mutations within a given cistron has already been carried out. The best example is that of the T^4 phage, parasitic on *Escherichia coli*, which has been studied by Benzer. The mutant of this phage, known as $r\mathrm{II}^{17}$ is characterised by its inability to lyse a particular strain, *K*, of *Escherichia coli*. Benzer obtained a considerable number of strains of phages $T^4r\mathrm{II}$ and compared them by a method comparable to that of Demerec. A culture of *Escherichia coli*, strain *K*, was simultaneously infected by two strains of $T^4r\mathrm{II}$ phages. When a bacterial cell is infected with two phages at the same time, exchanges of chromosome fragments can take place between them. Under these conditions, recombinants appeared growing on strain *K*. This is because the mutations carried by the two partners were not situated at exactly the same level on the chromosomes.

As in crossing-over in higher organisms, the nearer the chromosome alterations carried by the two strains are to each other, the more unlikely it is that reconstitution of an intact chromosome will occur. In consequence when they are close together, the active phages produced are rarer than when they are widely separated. Therefore a calculation can be made of the distance between two alterations, by recording the frequency of active phage produced when they are crossed.

This method has shown the existence of scores of distinct mutations inside the same cistron. Moreover, they have been found to belong to two different categories (fig. 14). Those of one group are rather unstable and show a high level of significant back-mutation, that is, they return readily to their initial state. A topographic study shows that they are point mutations of the order of size of the space occupied by a few atoms along the chain of an organic molecule. The other sort of mutations are rare and much more stable. They are shown by topographic study to correspond to a much more extensive alteration of the chromosome. The most generally accepted hypothesis regards such a mutation as a deletion whereas a point mutation would be a localised chemical change.

Structure of chromosomes

The chemical structure which ensures the transmission of the hereditary characters has been described in a previous chapter. Here,

(13) Zygote: *the product of the fusion of the male and female gamete. An egg which has just been fertilised is a zygote.*

(14) *The complete set of genes of an individual is described as the genome.*

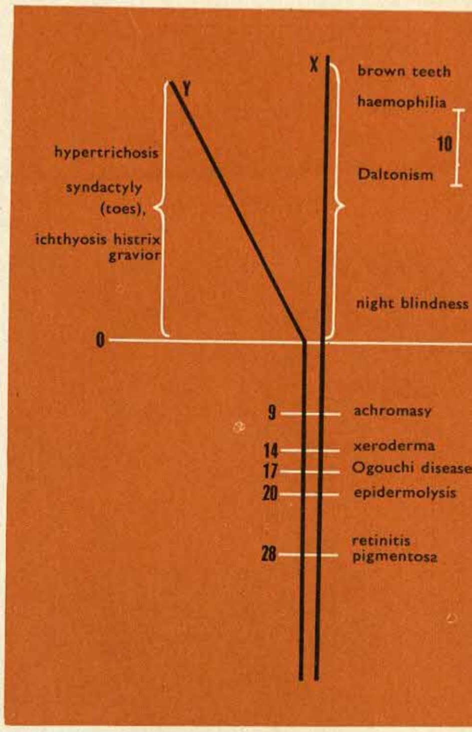

Fig. 13. Outline of the genetic map of the sex chromosomes of man. The female has two identical X chromosomes, carrying allelic genes. The male has an X chromosome and a Y chromosome. These two chromosomes have a homologous region carrying allelic genes and a differential region. In this region, Y is not homologous to X and it bears different genes which are not allelic to those of X. The brackets indicate the region within which the genes named in the diagram appear. Their relative positions are not yet known, except that on the differential segment of the chromosome, the genes for haemophila and for colour-blindness are 10 units apart and the one for night blindness is much further away. The figures along the homologous segments indicate the distance of the genes from the differential segment.

(15) Locus *(plural loci): the site, or location, on a chromosome of a particular gene or any of its alleles.*

(16) *The red colour of the eyes of albinos is a result of their depigmentation, which affects the iris as well as the hair, and lets the normal red colour of the retina at back of the eye be seen.*

I

(17) rII mutation: *When a smear is made of a bacterial culture infected by a virulent bacteriophage it develops 'plaques' on its surface, which are the zones where the bacteria have been lysed by the bacteriophage. The appearance of the plaques is often characteristic of the strain of bacteriophage, eg the letter r signifies 'rapid lysis'.*

(18) Histones: *slightly alkaline proteins, soluble in water and coagulated by heat. They contain 15 per cent of di-amino acids.*

(19) Antigen: *all those substances capable of inducing the appearance of specific antibodies when introduced in an organism.*

(20) Hyphae: *filaments without chlorophyll, which are the fundamental components of the body of a mushroom or a lichen.*

therefore, we will only show how the biochemist's conclusions agree with the genetical data. All modern research leads us to think of the chromosomes as made up of deoxyribonucleic acid (DNA) in association with a basic protein, usually a histone.[18] Without neglecting the role of the protein completely, there are good reasons to think that the essential role in the genetic processes is played by the DNA. Therefore an explanation for the mechanisms of heredity must be sought in the structure of this substance and the structure generally accepted today is represented by the Watson and Crick model.

This model represents the molecule of DNA as a double spiral composed of two long chains of complementary nucleotides twisted into helices. However, we know that only four types of nucleotide are present in this molecule and therefore the genetic information they carry is determined by the number, order, and sequence of these four types of nucleotide. Any alteration of this sequence will therefore constitute a mutation.

The smallest mutation which can be imagined, will result from the loss, reduplication, or exchange of a nucleotide. Thus the minimum distance apart between two distinct mutations, will be the distance between two successive nucleotides, that is 3·4 Å. But this distance is of the same order of size as that which has been found for the distance between the point, or reversible, mutations of the phages which would, in fact, be situated a few nucleotides from each other along the DNA molecule.

On the other hand, we have seen that the stable mutations of the phage correspond to a markedly more extensive alteration of the chromosome, and therefore involve more nucleotides. Thus, the simplest hypothesis is to assume that a small sequence of nucleotides has been lost. However, it has been known for a long time that a small chromosome deletion (loss of a short chromosome segment) produces exactly the same phenotypic effect as a factorial mutation and is transmitted in exactly the same way, so that it is impossible to distinguish one from the other. Also, long before the discovery of pseudo-allelism and the study of phage genetics, many geneticists believed that the factorial mutations were only chromosome mutations that were too small to be observed.

Thus, while these facts of a purely genetical nature do not categorically demonstrate the chemical structure of the hereditary material, they are in remarkable agreement with it.

Production of phenotypic characters

It has been known for a long time that there is a long chain of reactions between the gene on the chromosome and the phenotypic morphological or physiological characters which it controls. One of the best-known examples is that of the eye-colour of *Drosophila*, studied by Beadle and Ephrussi since 1937. This work is summarised in fig. 15. The initial material from which the brown pigment is synthesised is tryptophane, which is at first oxidised by means of enzymes into α-oxytryptophane and then into cynurenine. This oxidation takes place only in the presence of the gene v^+, and therefore does not take place in v/v individuals even when tryptophane is available. In these circumstances the eye has red pigment only and this is constantly produced. The red pigment (fig. 10) is brighter than that of wild flies, in which the eye colour results from superimposed red and brown pigments. The same applies to the transformation of cynurenine into chromogen. Here again, the enzyme action does not take place if the cn^+ gene is missing, although cynurenine may be abundant. This leads us to the conclusion that the primary action of the gene in both cases is on the production of an enzyme, and the ensuing processes are no more than a chain of biochemical reactions.

Today analogous reaction chains are known in various animals, but these mechanisms have been studied chiefly in man and micro-organisms. In man, many abnormal states occur, which derive from the inability of the body to carry out the transformation of a particular substance. By studying family genealogies, nearly all these cases have been shown to have a very simple genetical origin. Usually a recessive gene is involved, so that the genotype of normal individuals may be expressed by N/N or N/n and that of abnormal individuals by n/n. As with the *Drosophila* eye pigments just described, these abnormal characters result in most cases from failure in function of an enzyme mechanism.

Alcaptonuria provides a typical example. The urine of affected people darkens rapidly, because of the homogentisic acid which is eliminated in it. This substance does not occur in normal urine; for although it is produced from tyrosin during metabolism, it ordinarily undergoes an immediate enzymic oxidation which removes it. Therefore the principal characteristic of the affected individuals is the total absence of this enzymic mechanism for oxidation which leaves homgentisic acid to accumulate in the plasma from which it is eliminated into the urine. This character is genetically determined by the homozygous state of a rare recessive gene.

Serology has also provided many examples of simple genetic mechanisms, upon which the production, or absence, of some specific substance, is dependent. Each of the numerous antigens which characterise the blood of a given individual seem to be determined by the presence of a particular allele at a given locus.[15]

The four fundamental blood groups are determined by a system of three alleles I^A, I^B, I^o, which are capable of replacing each other at locus *I*. Therefore the four groups correspond to the following genotypes: Group *0*, I^oI^o; Group *A*, I^AI^A or I^AI^o; Group *B*, I^BI^B or I^BI^o; Group *AB*, I^AI^B. The allele I^o produces no antigen, while allele I^A produces antigen A, and I^B antigen B, in whatever genetic combination they are found.

Among the micro-organisms, such as the bacteria, yeasts, and fungi, a very large number of mutations are known, which usually take the form of the inability of a mutant to synthesize a particular metabolite and thus to grow on a medium which does not contain it. Such mutations are called 'biochemical mutants', and it is often very easy to discover them. For example, in the case of fungi many spores may be sown on a weak medium composed of water, mineral salts, glucose and traces of biotin. When cultivated on such a medium, the normal spores develop hyphae[20] and the mutants which need for their growth some substance missing from the medium do not grow. The normal spores are then easily isolated by simple filtration, since the hyphae retain them, leaving the mutants to be studied genetically and biochemically.

Thus, the examination of many examples, from such different fields as the genetics of man, *Drosophila* and micro-organisms, has led us to the conclusion that the primary action of a gene is nearly always the production of an enzyme (or some other specific substance such as an antigen)[19]; hence the theory 'one gene, one enzyme'.

Cytoplasmic heredity

Except in the very special case of the bacteriophages, it is not only the chromosomes that are passed on to the next generation, but an egg, which is a complete cell with a nucleus and sometimes a very abundant cytoplasm. While the nucleus apparently plays a leading part in hereditary mechanisms, the cytoplasm may, nevertheless, transmit other things besides a simple store of material destined solely to come under the control of the chromosomes. Hence the concept of cytoplasmic heredity.

However, while the true function of the chromosomes is to transmit the hereditary characters, a function for which they are especially fitted (thus explaining the remarkable uniformity of nuclear heredity which is linked to the uniformity of chromosome structure in all living things), the cytoplasm, on the contrary, has many other functions to perform and in some instances the processes of transmission that it brings about must be regarded as incidental. These processes are also very heterogeneous and the study of cytoplasmic heredity is much less advanced than that of nuclear heredity, so that it often seems a mass of unrelated phenomena.

The only truly universal characteristic of this type of inheritance is its lack of regularity. The male provides very little more than a nucleus, so that cytoplasmic heredity is primarily maternal. Whilst not pretending that it is possible to classify all the phenomena of cytoplasmic heredity, there is no doubt that many of them can be grouped into three categories: the inheritance of early developmental characters, the transmission of those cytoplasmic elements endowed with genetic continuity, and contagious heredity.

Numerous observations have shown that the first steps in individual development are determined much more by the cytoplasm of the egg than by the nuclear genotype, which only progressively takes control during the later stages of morphogenesis. In fact, in several cases the nucleus of an egg has been successfully cut out with a micromanipulator and the egg's development was found to continue for some time completely normally without any nuclear intervention. A much more simple method is to make a hybrid egg, so that the cytoplasm and nucleus are of type A and the sperm of type B. The fertilised egg which results will have a nucleus of type AB in a cytoplasm of type A.

In most cases the beginning of development is essentially of type A, that is, maternal, and sometimes the individual keeps the mark of this maternal developmental beginning throughout life. Such, for example, is the case of coiling in opposite directions in gastropods. In *Limnæa peregra*,[21] two races are known, one coiled to the right[22] and the other to the left. They differ from each other by a couple of

(21) Limnæa peregra: *The Limnæas are pulmonary gastropods, that is, they belong to the same group as the snail, but they live in fresh water and are very abundant in ponds. Their shell is more delicate than that of a snail.*

(22) Right-handed shell: *the shell of a snail and other gastropods is composed of a conical tube coiled into a helix. This helix may be coiled to the right or to the left. A corkscrew and a normal screw are coiled to the right. Many of the most widespread gastropods are coiled to the left.*

(23) Autosomic genes: *autosomes are the chromosomes which occur in pairs, in contrast to the sex or heterochromosomes. The genes found on the autosomes are autosomic.*

(24) Chloroplasts: *cellular inclusions containing chlorophyll. (Also called simply 'plastids').*

J

(25) Trypanosomes: *flagellate protozoa which generally have an elongated shape and are furnished with a single flagellum pointing towards the back. These parasites often have a complex life-cycle. Some are pathogens of man, the best-known one being* Trypanosoma gambiense, *the cause of sleeping-sickness transmitted by the tsetse fly.*

(26) Symbionts: *beings which live in symbiosis.*

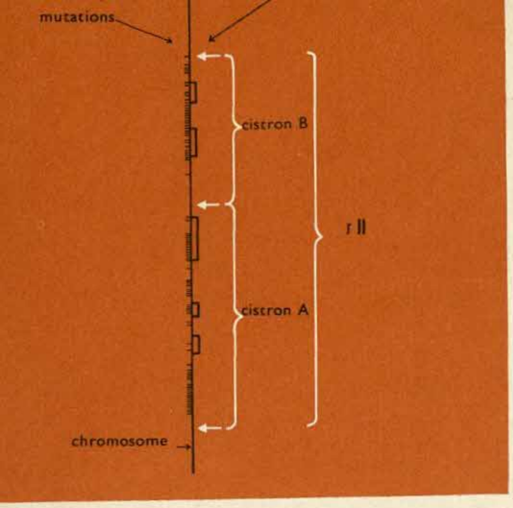

Fig. 14. Part of a chromosome of the T4 phage, parasitic on Escherichia coli, *according to Benzer (diagrammatic). The region rII where all the mutations producing the character 'inability to grow on type K' include two cistrons which correspond to two ways in which this condition is brought about. Inside each of these cistrons, a large number of point mutations can take place. These are represented here by the small horizontal projections to the left of the axis which represents the chromosome. A much smaller number of stable mutations, corresponding to an alteration of a longer chromosome region, is represented by the vertical lines attached to the right of the chromosome axis. Not all the mutations known today are shown in this diagram.*

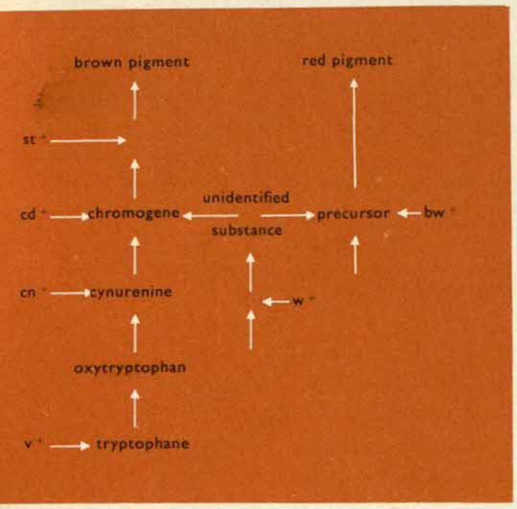

Fig. 15. Chain of reactions leading to the production of the eye pigments of drosophila.

autosomic[23] genes, with coiling to the right dominant. In all the crossings which can be made, the direction of coiling of an individual is determined, not by its true genotype, but by that of its mother.

Therefore, these observations, and several others, show that some of the characters of the first stages of development are determined by the genotype, not of the embryo, but of its mother; for the structure of the egg's cytoplasm is synthesized before it is fertilised and is under the control of only maternal genes. It is hardly necessary to attribute this type of heredity to the cytoplasm. Rather is it a very brief inheritance which only defers and precedes the action of the nuclear genes.

Besides this deferred nuclear heredity, true cytoplasmic heredity exists which involves the regular transmission from one generation to another of cytoplasmic particles which are incapable of arising *de novo* and come from the division of pre-existing particles of the same type being endowed with genetic continuity. This, for example, is seen in the case of plant chloroplasts[24] which multiply by division within the cell, in the form of plastids or proplastids. When the cell divides they are distributed between the two daughter cells. If *Euglena meslini* is grown in the dark, the speed at which the chloroplasts multiply can be reduced so that their numbers are progressively reduced; finally cells are obtained in which they are absent. These strains of Flagellates are incapable of regaining their chloroplasts.

Other cell inclusions comparable to the plastids of plants are known in animals, chiefly among the Protista, for example, the kinetosome of Flagellates and Ciliates. This little body situated at the base of the flagellum or cilium can only multiply by dividing into two and never arises *de novo* within the cell. It, too, is endowed with genetic continuity. The same phenomenon can be observed in the case of the kinetoplast of the trypanosomes.[25]

Finally, cases are known of a quite different kind of inheritance. This is contagious heredity. Briefly, it involves the infection of the organism at some stage during its early development by a foreign micro-organism and this process ranges from the simple, almost accidental, contamination of an embryo as hereditary syphilis, to the regular transmission of a symbiont,[26] where a self-reproducing particle becomes a part of the cytoplasm as with the *K* particles in the paramec *Paramecium aurelia*. In this species of Ciliate, there is a variety known as the 'killer' paramecium, producing paramecin, which destroys individuals of 'sensitive' strains, but to which they themselves are immune. If the cells of the killer strain are examined, about a thousand *K* particles formed of deoxyribonucleoprotein are found in the cytoplasm. These are not present in the cytoplasm of the sensitive strains. The genetic study of this phenomenon shows that the killer character is controlled by a nuclear gene, *K*, while the sensitive character corresponds to its allele *k*.

If a killer strain is cultured under suitable conditions, cellular division may take place more rapidly than division of the K particles. In this way the strain is gradually deprived of them and at the end of a certain period, cells without *K* particles are obtained, although they have the killer genotype. Such a strain will never again produce more *K* particles, under whatever conditions it is cultured. Thus the gene *K* does not provoke formation of the *K* particle, but renders it possible; their multiplication is by self-reproduction.

It is very difficult to say whether the particles are true cellular inclusions of the paramecium or whether they are a symbioti virus. The fact that they have been shown to mutate has been interpreted by some in favour of the hypothesis that the *K* particle is a virus which is only capable of multiplying in a paramecium of a particular genotype. But if so, it behaves as a very remarkable virus, protecting its host against events which are fatal to uninfected individuals . . .

L'Héritier has studied an analogous situation in *Drosophila*. This is the presence of a sensitive strain which is intoxicated by high concentrations of carbon dioxide, although this gas behaves as a particularly innocuous anaesthetic in the case of normal flies. A very detailed genetic study has been made of this character, which demonstrates that it is controlled by a cytoplasmic factor communicated by contamination, so that any fly can be sensitised by injecting it with an extract of sensitive flies. But in some strains, which are very common in nature, this character is transmitted in an extremely regular manner, so that, if it is the action of a virus, it can be said to be completely incorporated into the normal genetic mechanism of the flies upon which it is parasitic.

These few examples serve to show how varied and ill-defined the processes of cytoplasmic heredity are. They are also very confusing for geneticists accustomed to the quasi-mathematical rigidity of the mechanisms of nuclear heredity, and much still remains to be done in this field.

> The body alters shape constantly, or rather there is no shape, as shape is immobility and reality is movement; that which is real is the continuous change of shape: shape is but a snapshot of a transition.
>
> HENRI BERGSON

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PART FOUR

The main stages of development

We have seen how germ cells from individuals of different sex give birth to a fertilized egg, the cell which is a new living being. We shall now see the various stages through which the egg must pass to give an adult individual. The illustrations on page 129 show in the most expressive way how a vertebrate develops: Pleurodeles waltlii.

There is a series of periods each with its distinctive character. The beginning of development, called segmentation (figs. 1 to 5) is characterised by great mitotic activity; the successive divisions of the egg into 2, 4, 8, 16, 32, etc., daughter cells leads to the formation of a small hollow ball, made up of a single layer of cells, the blastula (fiig. 6). Then follow a series of important cellular migrations, gastrulation, during which the three layers—the endoderm, the mesoderm, and the ectoderm—fall into place (figs. 7, 8 and 9).

At the end of this period, the gastrula is completely covered by the ectoderm; the endoderm fills the centre of the embryo; the mesoderm is between the two. The following stages (figs. 10, 11 and 12) are called the neurula. It is during this period of neurulation that the first axial organs of the embryo are formed: the notochord and the neural tube. All other organs become differentiated during the fourth period (fig. 13), during which time the embryo lengthens considerably by proliferation of its caudal cells (fig. 14). After this stage, the embryo hatches, swims actively in the water, and is known as a larva (fig. 15); this is the larval period. Then the larva goes through metamorphosis to acquire, with the appearance of legs (figs. 16 and 17) and the disappearance of the gills, the final adult shape (figs. 18 and 19).

1. Undifferentiated egg; 2. Stage 2:2 blastomeres; 3. Stage 4:4 blastomeres; 4. Stage 8:8 blastomeres; 5. Stage 32:32 blastomeres; 6. Young blastula; 7. Gastrula: view of animal pole; 8. Gastrula: view of vegetative pole when blastopore appears; 9. Gastrula: view of vegetative pole at end of gastrulation, when yolk plug closes; 10. Neurula: medullary plate; 11. Neurula; 12. Neurula: closing of medullary folds. 13. Caudal bud stage, side view; 14. Early larva, a few days before hatching (taken from egg); 15. Larva hatched, bud of fore limbs; 16. Young tadpole, fore limbs lengthened; 17. Abnormal young tadpole (six months) just before metamorphosis; 18. Adult female of Pleurodeles waltlii *(newt); 19. Adult male of* Pleurodeles waltlii. *(The photographs on p. 129 were taken by permission of Professor Gallien in the Embryological Laboratory at the Faculty of Science in the University of Paris.)*

A giant cell: the egg

Jean CLAVERT

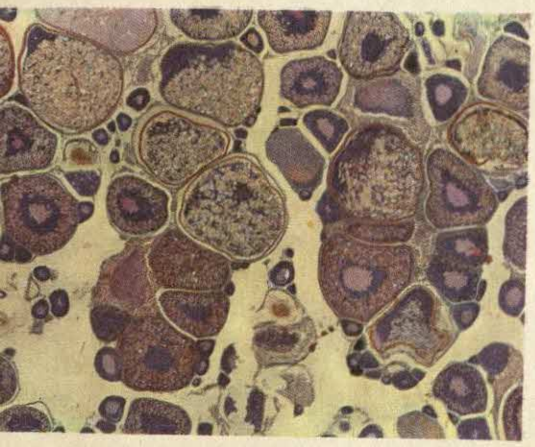

Fig. 1. Photomicrograph of a section of fish ovary (minnow), showing different stages in the development of the eggs. The central pink regions represent the germinal vesicles, the violet ones around these show the living protoplasm in which yolk vacuoles progressively appear, representing the inert reserve substances (mag. × 10) (photo Clavert).

A fertilised egg is a cell which contains the potentialities for developing into an individual identical with its parents.

Although the dimensions of eggs vary enormously, they are always very large and usually spherical cells. Their size is practically constant for any particular species, but varies widely between species, ranging from a tenth of a millimetre in mammals and many invertebrates, to several centimetres, as in a hen's egg, where the yolk is some 3 centimetres across. Only what is contained within the membrane that closely surrounds the yolk is the true egg; the white and shell are membranes.

Like all cells, the surface of the egg is bounded by a cell membrane. In the egg this has a special name, the vitelline membrane. The cell also has a nucleus, cytoplasm, cell inclusions responsible for synthesis, and a variable amount of reserve material, the yolk. The mass of yolk is largely responsible for the size of the egg. Thus, the amount of yolk present forms the basis of the most frequently used classification, which distinguishes between three main types of eggs (fig. 3):

1. *Oligolethical eggs*, which contain very little yolk and have diameters of the order of 0·1 millimetre. This kind of egg is produced by Sponges, Coelenterates, Echinoderms, *Amphioxus* and mammals.
2. *Lecithical eggs*, having plentiful yolk which tends to accumulate at one pole. The eggs are of the order of one millimetre across and are produced by Molluscs, Annelids, Amphibians and some fish.
3. *Telolethical eggs*, which have a large amount of yolk and diameters measured in centimetres. Reptiles, birds and some fish produce this type of egg. Whatever the type of egg, it has special morphological characteristics which distinguish it from the other cells of an animal.

The nucleus of a fully-developed egg is at least two or three hundred times larger than an ordinary nucleus. It is large and clear and is called the germinal vesicle. This plays an important part in synthesizing the cell substances and certainly produces those which give the egg its special properties. The nucleus also takes part in the determination of many of the hereditary characters.

The cytoplasm forms a special layer called the cortex at the periphery of the egg and encloses the yolk. It is mainly made up of proteins which differ in their physico-chemical nature from one species to another. This cortex is probably the site of many of the formative potentialities of the egg; it is *par excellence* the living component of the cell. The arrangement of these proteins, the changes which they undergo, and the relationships they enter into with the other underlying parts play a fundamental role in the mechanism of embryonic development.

The yolk is an inert structure which is arranged in the egg in a clearly defined way, giving the egg of each species its characteristic appearance.

The nucleus of a ripe egg is always eccentric, so that the egg has an axis of symmetry represented by a straight line joining the nucleus to the geometric centre of the egg.

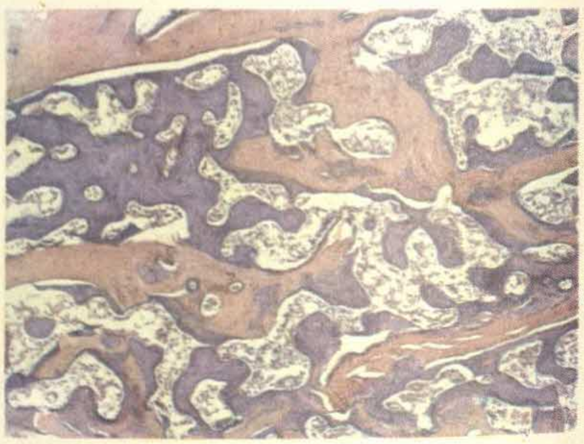

Fig. 2. Photomicrograph of a section of a laying duck's tibia. The areas of compact bone are pink, the violet region represents the follicular bone with the special function of forming the shell of the eggs. This kind of bone is only found in laying females and is formed by the action of ovarian folliculin (mag. × 68) (photo Clavert).

Among the most interesting problems of biology is yolk formation and storage and the development of the structural basis of the egg's potentialities. Yolk formation takes place during a special period in the life of the egg: the growth phase. The whole of this stage takes place in the ovaries, which are specialised organs where the egg is situated in a suitable

(1) Follicle: *organ in the form of a small sac.*

(2) Chondriome, *Golgi substance, ergastoplasm: See p. 59 'From cell to tissues'.*

(3) Radial symmetry: *symmetry revolving round an axis.*

(4) Preformation: *theory according to which all future differentiation of the embryo is preformed within the egg (Bonnet and Haller theory).*

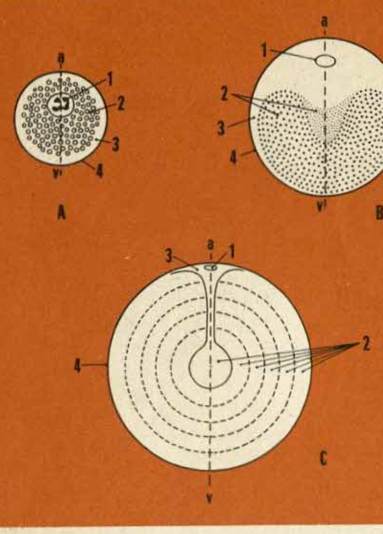

Fig. 3. Diagrammatic representation of three kinds of vertebrates' eggs (axial section). (1) shows the nucleus or germ vesicle; (2) the reserve substances or yolk; (3) the cortex and (4) the egg membrane or vitelline membrane. The nucleus is always eccentric in the egg and its position defines the animal pole (a); the vegetative pole (v) is opposite. In these three cases, the axis av represents a symmetrical axis for the egg round which the various components are evenly spread. A *represents an isolecithal egg of a mammal (real size 0·2 mm diameter);* B *a mesolecithal egg of an amphibian (real size 2 mm);* C *a telolecithal bird's egg (size 1 to 3 cms).*

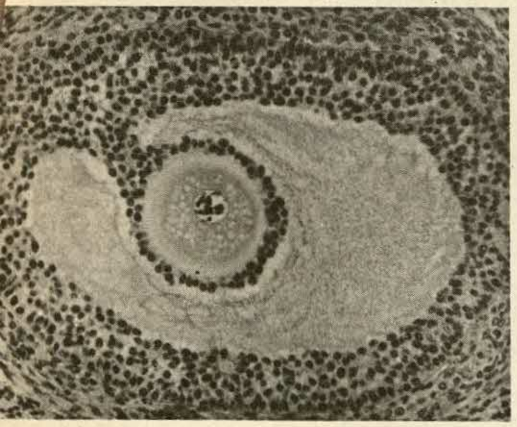

Fig. 4. A mammal's egg (rabbit) in its follicle. Size of egg and nucleus can be compared to the size of the follicular cells surrounding it. These are normal size cells, the egg being, in fact, a giant one. The human egg looks exactly similar (mag. × 180) (photo Clavert).

environment for its formation. It lies in the dense body of the ovary, surrounded by several layers of organised cells known as follicle cells, which enclose the egg as in a pouch. The egg and its follicle cells together constitute a follicle[1] (fig. 4). Among mammals, the follicle cells form a large mass which breaks up to form a cavity which becomes filled with fluid. Each follicle is surrounded by a dense network of blood vessels, which supplies nourishment for the egg via the follicle cells. It is also surrounded by numerous endocrine cells which secrete, at intervals determined by the rhythm of the pituitary gland, a hormone called oestradiol, which plays an important part in the growth of the egg (fig. 1).

The formation of a bird's egg will be taken as an example. In this, egg yolk production takes place in two distinct phases. First a period of slow, then one of rapid growth.

In birds, as in all other vertebrates, the phase of slow growth begins the moment the bird is hatched and lasts for several months. During the whole of this long period, the young egg possesses numerous synthesizing components spread throughout the entire cell, namely a huge nucleus, mitochondria, Golgi apparatus and ergastoplasm.[2] Either under their action, or by direct transformation, the cytoplasm and the first dilute droplets of yolk are slowly formed. This process of yolk formation takes place unobtrusively inside the ovary, without repercussions on the rest of the body. At most, it results in the formation of 5 per cent of the fully developed egg. However, it is during this period that the special cytoplasm of the egg, with its great potentialities, is built up.

The phase of rapid growth follows the preceding stage after a variable delay and results mainly in the accumulation of inert reserve substances. In its intensity and the upset that it brings about in the female body it contrasts sharply with the preceding phase. It lasts from four to fourteen days, according to the species, and it is during this period that 95 per cent of the yolk of the egg is formed. This yolk is concentrated and mainly composed of proteins (chiefly phosphoproteins known as vitellins) and lipids (various fats). In order to bring about this stage, important changes in the blood are necessary. Indeed, at this time, the level of almost all the blood constituents is observed to increase, often by as much as ten times, and vitellins, which are not normally found in the blood appear. Therefore, during this period, the yolk substance is drawn directly from the blood.

The transformation of the egg

The signal for the onset of rapid growth is dramatically provided by the sudden release of a hormone by the pituitary gland. This secretion is itself initiated by internal and external factors, such as temperature and illumination, and even by psychological factors. On reaching the ovary, this hormone acts on the endocrine cells surrounding the follicles.

The follicles then begin to secrete oestradiol, which causes the neighbouring blood vessels to dilate and increase in permeability. This hormone then passes into the blood and stimulates many organs. among them the liver, which begin to make special substances for the egg, and release them into the blood. These are selectively extracted by the follicles whose blood vessels have been dilated and made more permeable. The follicle cells are bathed by a transudate from these blood vessels from which they fix the materials and pass them on to the egg to be stored. Other substances are added to this store; they come directly from the digestive tract and enter the egg in the same way.

The yolk thus accumulated, forms a reserve which must provide for the needs of the developing embryo. Part will be used to build up the tissues of the new animal directly, and the rest will provide the energy required to convert the reserves into embryonic tissue.

An egg is often covered by membranes formed in the genital tract. These serve to protect the egg and also to supplement its reserves. For example, in a hen, the white of egg and the shell act as a reserve of water and calcium from which the embryo draws.

Oestradiol also acts on the female skeleton to make a special type of porous bone which serves solely as a reserve of calcium for the formation of the egg shells (fig. 2).

In order to appreciate the exertion demanded from a hen while laying eggs, one only has to realise that in a month it produces its own weight in eggs, and, during the same period, uses up as much calcium as is in its whole skeleton to provide for the formation of the shells. All the lower vertebrates, fish, amphibians, reptiles and birds, have to make a similar effort during the stage when their eggs are undergoing rapid growth. They are able to do so on account of the action of the ovarian hormone, oestradiol, which puts the whole female organism in readiness to provide for the enormous extra requirements.

Among mammals, which lay a small number of oligolethical eggs, yolk formation becomes insignificant. The energies of the female go to building up the foetus rather than to forming the egg.

An egg is a giant cell which contains *in posse* the entire individual to be. Biologists, in years to come, will have to clarify the exact basis of the egg's potentialities and the manner in which they are expressed.

The problem of bilateral symmetry

The appearance of an egg is that of a sphere or oval, with its different constituents arranged around an axis of symmetry. At the ends of this axis two poles may be distinguished. One is the animal pole, which is also the point on the egg's surface nearest to the nucleus. The other is the vegetative pole where the heavy reserves accumulate. In all vertebrates this radial symmetry is converted into a bilateral symmetry in the course of development. Any median plane of the egg may become the bilateral plane of symmetry, dividing the egg into the right and left sides of the future animal. The head-tail (cephalo-caudal) axis of the body is situated along this plane, with the head always near the animal pole (fig. 5).

For a long time the 'Preformationists' believed that the future differentiation of the parts of the embryo was already fixed in the egg. The results of experiments by Jenkinson, Newport, A. Brachet, Ancel, Vintemberger and Clavert have proved that the situation is quite otherwise, for the formation of bilateral symmetry in the egg is determined by external factors, although the mechanism of determination varies according to the type of egg. This phenomenon has been studied in amphibians and birds and it is now possible for us to understand some of these mechanisms more clearly.

The egg of the common or brown frog is a small sphere about two millimetres in diameter. It has two clearly differentiated poles: a white vegetative pole and a pigmented animal pole. The pigmented cap extends from the animal pole to just below the equator, where it ends in a transitional grey zone at the same level all round. The egg has other natural markings which are invaluable to the investigator. Exactly at the position of the animal pole there is a clear spot, the upper pole spot, which contrasts clearly with the dark surrounding background. Conversely, at the lower pole, there is a deep black sub-cortical[5] spot, the lower pole spot.

In axial section the egg is seen to have a thin peripheral layer of cytoplasm, the cortex. This is strongly pigmented and gets thinner from the animal pole towards the vegetal pole. The centre is occupied by a compact mass of yolk on the side of the vegetative pole and by cytoplasm on the side of the animal pole. The nucleus is found in the middle of this cytoplasm. Everything is regularly arranged round the axis of symmetry: the most detailed examination shows nothing to suggest the least heterogeneity.

However, one or two hours after fertilisation, at 18°c, the structure of the egg undergoes an upheaval and a special structure, the grey crescent[6] (fig. 6) is formed. From then on, the egg is bilaterally symmetrical. The grey crescent marks the position of the future dorsal surface of the embryo. Its centre indicates the plane of symmetry of the body that will develop.

What has happened? The work of Jenkinson, Ancel and Vintemberger explains this. It showed that what is brought about in the egg is a movement, which consists of the cortex sliding on the underlying layers. In the course of this rotation, the peripheral layer is displaced by an amount which is fixed for each species, for example 30° for the frog and 15° for the newt. The extent of this movement decreases from the plane of symmetry towards the sides of the egg where it becomes nil. The lower pole spot remains in place and enables us to gain some idea of the degree of displacement. In the dorsal hemisphere, the thickness of the pigmented layer decreases and one zone, the grey crescent, loses its pigment, which moves to the opposite side. From this stage onwards, the position of the future organs is irreversibly determined. The process of morphogenesis in the egg has begun.

Under natural conditions, the plane of bilateral symmetry of the future tadpole is determined by the point of penetration of the sperm, which may occur at any point, and the axis of radial symmetry. This determination depends, therefore, on an external factor. Other external factors may also have the same effect. For example, rotating the egg within its coverings, compressing it between two parallel surfaces, putting the egg in a constrained position or with an inclined axis, and so on. All these have the same effect as the sperm and may be substituted for it.

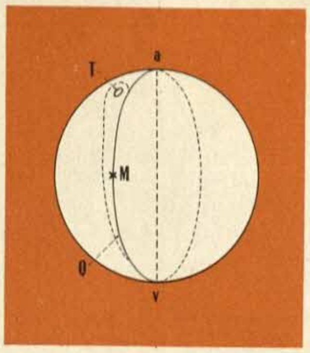

Fig. 5. In this diagram, av *represents the symmetrical axis of the egg;* aMv *the meridian which determines, with the axis* av, *the bilateral symmetrical plane of the embryo. The cephalocaudal axis of this embryo will be found along the meridian* aMv *and the head is always near pole* a *(animal pole). The structural polarity of the egg ensures this position. This diagram shows how the radial symmetry of the egg develops into the bilateral symmetry of the embryo.*

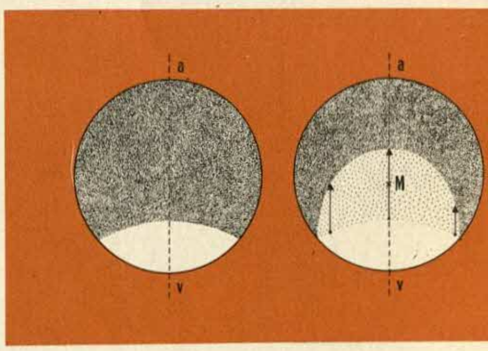

Fig. 6. Diagrammatic representation of an unfertilized frog's egg on the left and a symmetrically arranged egg, with grey crescent formed, on the right. The region bearing the grey crescent becomes the back and the middle of the crescent M *shows the position of the bilateral symmetrical plane. Arrows show the direction and amplitude of the movement of the cortex, which brings about partial depigmentation of the region and the formation of the grey crescent and symmetrisation of the egg.*

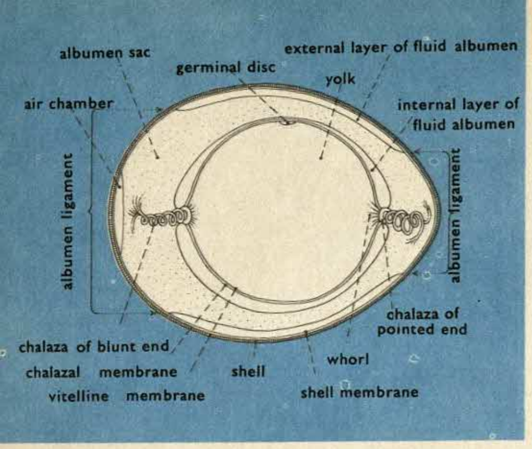

Fig. 7. Diagrammatic representation of axial section through a bird's egg. This diagram shows the movement undergone by the egg in the oviduct: the membranes (albuminous sac and shell) revolve round the yolk or egg proper, which remains balanced almost stationary in the internal albuminous liquid layer. A twisting of the base of the chalazae results.

(5) Sub-cortical: *the region just beneath the membrane of the egg.*

(6) Grey crescent: *See p. 133*

(7) Sub-germinal cavity: *small cavity under the blastoderm (see chapter 'How the embryo is formed'), so that the latter is only in contact with the vitellus on the edges.*

(8) Organising centre: *See chapter 'The embryo takes shape', p. 133.*

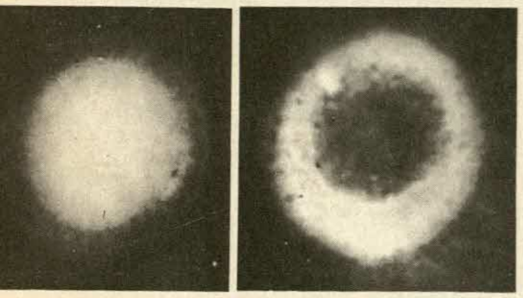

Fig. 8. This figure shows two germinal discs, the one on the left from a hen's egg to be laid eight hours later, the one on the right taken from an egg soon after laying. The first is still homogeneous, the other shows an eccentric inner zone formed shortly before. This brings about the symmetry of the egg (photo Clavert).

At all events, the only effective external factor is the last one to act on the egg before the rotation of fertilisation begins. Apparently, the external factors have a preparatory effect in orientating the rotation, but it is the rotation alone which determines the position of the plane of bilateral symmetry. Nothing in the frog's egg is fixed before rotation, whereas after it has taken place everything is determined. Fixing takes place in a few seconds.

The development of symmetry in birds

We will now see how the plane of symmetry is produced in birds. First, it must be borne in mind that all bird's eggs are constructed according to the same plan. In the centre is the yolk, or, strictly speaking, the egg, surrounded by its membranes. These are the shell and the albumen, or white of egg, forming an albuminous sac to attach itself to the shell. On the surface of the yolk a small clear patch appears: this is the germinal disc. It is situated under the membrane which encloses the yolk and marks the animal pole and one of the ends of the axis of radial symmetry of the egg. The other end of the axis is the vegetative pole, but there is nothing visible to mark its position.

The yolk floats, germinal disc uppermost, in a surrounding fluid mass attached to the albumen by two cords, the chalazae. These chalazae are rolled up in opposite directions, forming an axis round which the egg can turn to preserve its position of equilibrium.

The membranes of the egg are formed in the female genital passages, during its journey through them, which takes an average of twenty to twenty-two hours in the hen. The egg stays longest in the lower part of the oviduct, where it picks up water and forms its calcareous shell. While it is in the oviduct, the egg rotates in jerks, for sixteen to eighteen hours. The action of the oviduct's muscle brings about this slow rotation at a rate of about ten to fifteen turns per hour and always in the same direction. Only the membranes turn, the yolk remaining in the centre in equilibrium, almost immobile. This results in the chalazae being rolled up. They twist back upon themselves in opposite directions, one clockwise and the other anti-clockwise.

If a very young bird embryo is examined as soon as it is possible to distinguish the head and tail end, it is seen that there is a strict relationship between the orientation of the embryo, the longitudinal axis of the egg, and the way the chalazae roll up. The left chalaza is always found on the right of the embryo and the right chalaza on the left. The movement which results in the rolling up of the chalazae takes place in the tail-to-head direction of the embryo. Thus it is easy to think of the effect which the rotation of the egg has on the orientation of the plane of symmetry, but it had yet to be demonstrated.

By a series of x-ray photographs, useful information was to be provided by studying the development of birds' eggs in the oviduct and, in fact, it is possible by this means to show that the orientation of the embryo is a function of the position of the egg in the oviduct. If its position is changed experimentally, it is possible to influence the future orientation in a fixed and predictable way, and this can even be brought about *in vitro* by a special apparatus.

In a bird, as in a frog, there is a time after which all such changes are without effect. This critical phase is also accompanied by a sudden change in the germinal disc, which appears in the hen some six to eight hours before laying and is characterised by the formation of a clearer region excentrically in the germ. This region forms the outside of the subgerminal cavity[17] which forms at this time. The blastoderm is only in contact with the yolk at its edges. From then on the symmetry of the egg is fixed. Probably it is brought about by the membranes rubbing on the germ and as a result the orientation is a function of the position of the egg in the oviduct (figs. 7 and 8).

These two examples illustrate the importance of the action of external factors in fixing the plane of bilateral symmetry. The investigator can impose a particular plan of symmetry upon the egg of a frog by arranging for the spermatozoa to enter at a selected point, or upon the egg of a bird by altering its position in the oviduct. He can direct the changes in the structure of the egg, but cannot influence the subsequent reactions: as yet he has no knowledge of the exact direction of these. A given egg reacts invariably in the same way to external factors governing symmetry. This decisive stage of development determines the position of the organisation centre,[18] and from that, the normal position of the organism's layout.

The embryo takes shape

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Individual development or ontogeny in amphibians takes place outside the mother, and thus it is an easier matter for scientists to follow the course of development as it occurs. In the amphibian development, it is Nature itself that provides the opportunity for an embryologist to investigate the ontogeny of the vertebrates. In the main, the embryonic development of all vertebrates is in fact very much the same as that to be observed in amphibians. Experimental investigation of amphibian development has brought to light the existence of many factors which serve to direct the ontogeny of the vertebrate embryo into a harmonic whole, a new adult individual, as a result of the differentiation of cells and the structure of tissues.

In the spring, when the water in the pools has reached a certain temperature, the nuptial ceremonies of the amphibians begin. These result in the formation of masses of fertilised eggs coated with jelly. To the naked eye, these eggs appear as dark coloured spherical balls. If one of these lumps of jelly is turned over, it is possible to see the whitish underpart of the eggs; this cannot be observed for very long, however, as the heavier lower part of the eggs always brings them down.

Within the course of a few days, the eggs assume a more oval shape, and in two weeks or slightly longer each of them develops into a tadpole, which swims around in a lively fashion and, in fact, spends some weeks enjoying a life which is very much akin to that of a fish. By the autumn, tadpoles have undergone a process of metamorphosis,[1] lost their tails and gills, and taken up life on land.

From blastula to neurula

If the development of the amphibian egg is followed by means of a microscope, it will be seen that the egg first divides into two daughter cells; each of these subsequently splits into two, and so on. When this process of division has progressed sufficiently far, there is formed a ball with some hundreds of cells, which is known as the blastula. The upper part of this blastula, the so-called 'animal field' or 'animal hemisphere', in all species of amphibia is darker than the lower part, the 'vegetative field' or the 'vegetative hemisphere', which is milk-white in colour. On the equator of the blastula, there is a 'marginal zone' between the two hemispheres. In many amphibian species, this differs in colour from the other parts of the blastula. It is also broader on one side than the other, and is, by reason of its shape and greyish colour, also referred to as the 'grey crescent'.

A cross-section of the blastula reveals a large internal cavity, the blastocoel, which is lined with a layer of cells known as the blastoderm. In the animal field, the cells in this region are much smaller and the blastoderm itself much thinner than in the vegetative field, where the cells contain a considerable quantity of yolk. Consequently, the cavity is in the animal half of the blastula.

Gastrulation

After the blastula stage of development, there ensues a very complicated period, that of gastrulation. For a very long time genuine understanding of this phase was prevented by many difficulties, and a full explanation was not obtained until the 'vital dye' method was applied. This method consists of pressing small lumps of agar, impregnated with different kinds of vital dye, against the outer surface of the blastula. Ordinarily, the two dyes employed for this purpose are neutral red and Nile blue.

This method was discovered in 1923-29 by the German embryologist Walter Vogt and it has since been applied to many vertebrates other than amphibia.

The dyed agar colours the cells of the outer surface of the blastula without causing any damage and the coloured patches can then be followed through later development, and located after gastrulation (fig. 1).

During gastrulation, the blastoderm folds in such a way that eventually three layers of cells, one within the other, are formed. From the illustrations below it can be seen how this development takes place as shown by the vital dye method. The animal field (blue) forms the ectoderm, the vegetative field (yellow) the endoderm, and the marginal zone, or grey crescent (red), the mesoderm.

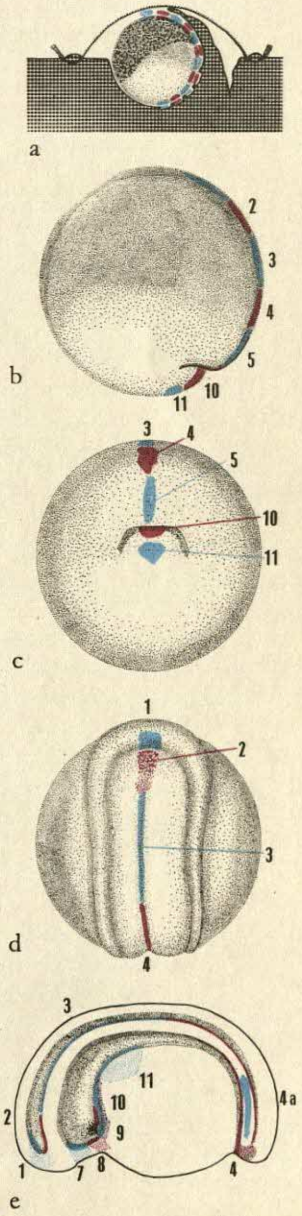

Fig. 1. Vital staining. (a): the device for putting coloured marks on a blastula; these are placed on the meridian through the grey crescent. (b and c): at the young gastrula stage; (b) side view, (c) from the vegetative pole. 6 to 9 are already invaginated, 1 to 5 drawn out. (d): back view of the neurula stage; 1 occupies the front medullary fold; note the lengthening of 2 and 3. (e): old neurula after neural tube has closed; 7 to 11 appear again on the base of the archenteron.

An indication of the beginning of gastrulation is a low depression, later becoming a horizontal slit, which develops on the surface of the blastoderm. It is on the borderline of the grey crescent. This depression is known as the blastopore. It rapidly develops in such a way that the future mesoderm, or so-called presumptive[2] mesoderm in its upper lip, becomes tucked in over the edge of the lip. Subsequently the presumptive endoderm below the blastopore follows the mesoderm primordia into the embryo.

This movement of the tissues is known as invagination, although strictly speaking, this term is not correct, as the word really means a substantial in-folding of a sheet of tissue. In fact, the movement of the mesoderm and endoderm primordia is no infolding at all, but rather a flowing or streaming movement of the cells. This can be seen from the elongation of the dyed areas which were originally rounded.

The invaginating stream of presumptive mesodermal cells starts in the middle of the blastoporal lip, but gradually broadens to both corners of the blastopore. Simultaneously, both corners grow along the border line between the presumptive mesoderm and the endoderm. After a while, the blastopore is sickle-shaped, and later on it assumes the form of a horseshoe; finally, it is circular, surrounding the part of the presumptive endoderm known as the yolk-plug which has remained on the outer surface of the embryo. In the next stage, the blastopore circle diminishes in size, the yolk-plug is completely swallowed, and eventually the blastopore is merely a narrow vertical slit. In the meantime, the presumptive mesoderm has continued its progress round the margin of the blastopore and the final result is that the whole of the grey crescent has disappeared from the outer surface into the embryo. The stage of development from the appearance of the first small blastopore depression to the disappearance of the yolk-plug is called gastrulation; by this time the ectoderm is covered entirely by the depression.

This is what can be observed as far as the outside of the gastrula is concerned. But what is happening simultaneously inside it? As the blastopore grew deeper, a hollow was formed, known as the primitive gut or the archenteron. During the process of invagination, the archenteron becomes longer, and soon its front wall touches the inner surface of the ectoderm opposite the blastopore. Subsequently, in the early larval stage, the mouth will be formed here, and later, the blastopore will be replaced by the anal opening.

When the primitive gut first begins to elongate, its roof is formed by mesoderm and its floor by endoderm; on each side there are the border lines of these two primitive layers. Originally the cavity of the early gut is a very narrow horizontal slit, but somewhat later, after its prolongation, the mesodermal roof splits away from the endodermal floor on both margins at the sides. The connection between mesoderm and endoderm remains permanent only at the front of the archenteron.

After the margins of the mesoderm have separated from the endoderm, they grow downwards between the covering ectoderm and the free margins of the endoderm. In the late stage of gastrulation, with a circular blastopore, the mesoderm surrounds the endodermal yolk-plug completely at the rear end of the endoderm. At the same time, the cavity of the anterior part of the archenteron becomes larger. In this area, the endoderm resembles an open trough over which there has been thrown a mantle, the mesodermal cell layer. Thus after gastrulation, the three germ layers are in their definitive positions.

Appearance of the nervous system: neurulation

When the yolk-plug has finally disappeared, the embryo is still almost spherical in shape; it is also evenly coloured by the dark pigment which is characteristic of the ectoderm. Shortly after this, there can be observed from the outside of the embryo the first signs of differentiation of the ectoderm. On the back of the embryo, starting from the blastoporal slit, we can see a slight longitudinal furrow and around this there will be formed a somewhat flattened pear-shaped area slightly darker in colour. This is the earliest appearance of the central nervous system which is at this stage known as the neural or medullary plate. The edges of the neural plate soon become elevated in ridges, the neural folds. Meanwhile, the spherical form of the whole of the embryo has changed; it has now become oval or slightly pear-shaped. With the passage of time, the neural plate narrows, especially at the blastoporal end, whilst the neural folds become higher, until it would be more appropriate to refer to the whole of the neural area as the neural groove rather than the plate. Finally, the neural folds come so close together that their margins touch and fuse one with the other; the neural tube has been formed. Eventually, it comes to lie beneath the ectoderm which covers it without a break.

(1) Metamorphosis: *end of larval period, start of adult life.*

(2) Presumptive: *qualifies the future differentiations of a territory or primordium of an organ when still indeterminate.*

(3) Notochord: *Cellular rod formed by the middle band of the mesoderm round which the spinal column differentiates itself.*

(4) Blastomere: *One of the cells derived from segmentation of the egg early in development.*

(5) Determination: *process during which the destiny of an embryonic territory is decided.*

(6) *For his experiment, Spemann perfected a special microsurgical technique, still used today in all laboratories. The egg is first freed of its gelatinous covering membrane by means of watchmakers' forceps. Spemann made new instruments to be able to cut up, take out and transplant small fragments of embryonic tissue: a pointed glass needle, a micro-pipette and a loop of a child's hair inserted into the end of a glass rod. For reasons of asepsis, these instruments have been replaced nowadays by a platinum needle and loop.*

(7) Self-differentiation: *Power of a primordium to differentiate.*

(8) *The implantation of killed tissues or inert substances in the young gastrula was only possible after a new grafting method was perfected by Otto Mangold, one of Spemann's pupils. The graft on the anterior side only succeeded if the graft was alive and at the same stage of development as the host. The new technique consists in introducing the implant into the blastocoele through a small opening in its top. During gastrulation, the implant is applied against the inner side of the ectoderm and can then develop in the same way as in the normal field. The cut in the ectoderm heals in a few minutes and the scion cannot be rejected (see fig. 2).*

(9) Thermolabile: *tending to decompose or become inactive on being heated.*

The stage of development from the first appearance of the neural plate until the closing of the neural tube is known as neurulation, and the embryo at this stage as the *neurula.*

Sections of the young neurula show that the neural plate is thicker than the rest of the ectoderm and when the neural groove is closed, its anterior part is very much larger than the rear. The anterior part will develop into the brain, and the narrow hind part will become the spinal cord. The greater part of the rest of the ectoderm eventually becomes the epidermis of the skin with its glands, its sense organs, or parts of them, such as the nose, inner ear, the lens of the eye. The light-receiving part of the eye is formed by a thrusting outward of part of the brain.

At the beginning of the neurula stage, the archenteron is still trough-like until both of its margins converge and fuse in the middle line. By this time the primitive gut is a blind tube, with a very thin roof, whereas the floor, with large cells rich in yolk, is singularly thick. Later in the larval stage, the endoderm will form the inner surface of the digestive tract with its glands. The lungs and large glands connected with the digestive tract, are also derived from the endoderm.

When the neural plate starts to differentiate, the mesodermal mantle splits into five longitudinal cell strips. In the mid-line beneath the neural plate, the first separation that takes place is of a narrow rod of cells, the notochord or primitive backbone.[3] Meanwhile, the strips of the mesoderm mantle, to the right and to the left of the notochord, are thickened and later, these will become isolated from the rest of the mesoderm and divide into a number of cube-shaped blocks of cells, the somites, which lie in a row on either side of the notochord. These are the source of the body muscles and the skeleton. The remaining margins of the mesodermal sheet divide into two layers, between which the body cavity is formed. The inner layer is later joined to the primitive gut, and forms the smooth muscles of the intestines and the connective tissues of the digestive tract. On the other hand, the outer sheet joins the somites to form the connective tissue of the rest of the body.

The problem of determination

Before the end of last century when we began to acquire knowledge of the main features of vertebrate ontogeny, the question often arose as to why the embryonic cells, originally all alike, were later differentiated into various types of tissue. Theories were suggested at that time in an effort to solve the problem. Among these was the 'preformation theory' proposed by August Weismann.

According to Weismann's theory, ontogeny was determined in advance, at an early stage in the fertilised egg; i.e., in every blastula each cell had a predetermined role in the future development. The young embryo would be rather like a mosaic in which every piece has its part to play in the whole.

This theory was purely and simply an idea which had no empirical background and we have to thank another German biologist, Wilhelm Roux, known as the father of experimental embryology, for bringing the problem of differentiation on to an experimental basis. He made the classic experiment of killing one of the blastomeres[4] at the two-cell stage of an amphibian in order to follow what he believed would be the independent development of the other living blastomere.

The result of this experiment at first seemed to support Weismann's theory, since the living cell did give rise to a half of the blastula; subsequently it was shown that this was merely the consequence of the remnant of the killed cell preventing the free development of the living one. When the cells of the two-cell stage were separated one from the other by a gradually-tightened loop of hair, it was soon realised that each cell at this stage was still capable of developing into a complete embryo, and even into an adult individual. Thus a fertilised egg, whose original function was that of developing into only a single individual, had produced twins. More recently it has been found possible to divide the blastula and the early gastrula into two parts, with the same results, provided that the ligature is placed along the middle line of the grey crescent.

These experiments served to show that in the early stages of development differentiation has not yet occurred, and that the cells of a young embryo have more capacities than would seem to be implied by their normal fate.

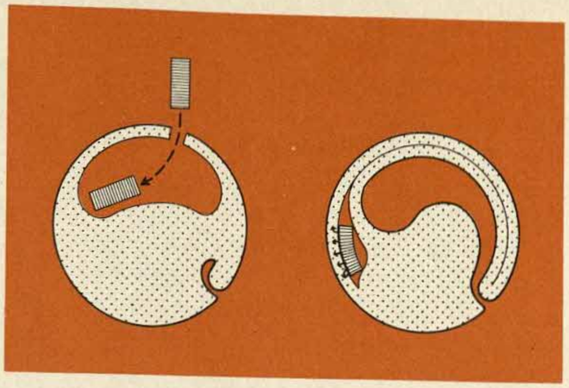

Fig. 2. (Left) means of inserting a graft to study the morphogenetic properties in the blastocoel of a young gastrula. (Right) after gastrulation, the graft is applied against the ectoderm, on which it can exercise its possible action (symbolised by tiny arrows).

HISTORICAL NOTES

1892
AUGUST WEISMANN gave his theory on the preformation of the embryo in the egg.

1893
WILHELM ROUX began experimental research on the development of vertebrates.

1895
ENDRES obtained twins by binding together frog's embryos at the two blastomeres stage with the help of a hair.

1901–1912
HANS SPEMANN perfected microsurgical methods. He discovered the determination stage of various parts of the blastoderm. He also established the relationship between the secondary optic cup and the crystalline lens.

1923–1929
WALTER VOGT made a map of presumptive primordia of amphibian blastoderms by the method of vital staining.

1929
OTTO MANGOLD invented the insertion method which consists in implanting a graft in the blastocoel of a young gastrula.

1932
SPEMANN, BAUTZMANN, HOLTFRETER and O. MANGOLD published a report on the chemical nature of the primary action of the organiser.

1932–1936
Teams of research workers tried to discover the chemical nature of the organiser.

1938
CHUANT and TOIVONEN published a report showing that some adult tissues exercise a primary specific action on ectoderm. From this observation followed the present-day view that differentiation is due to several chemical substances.

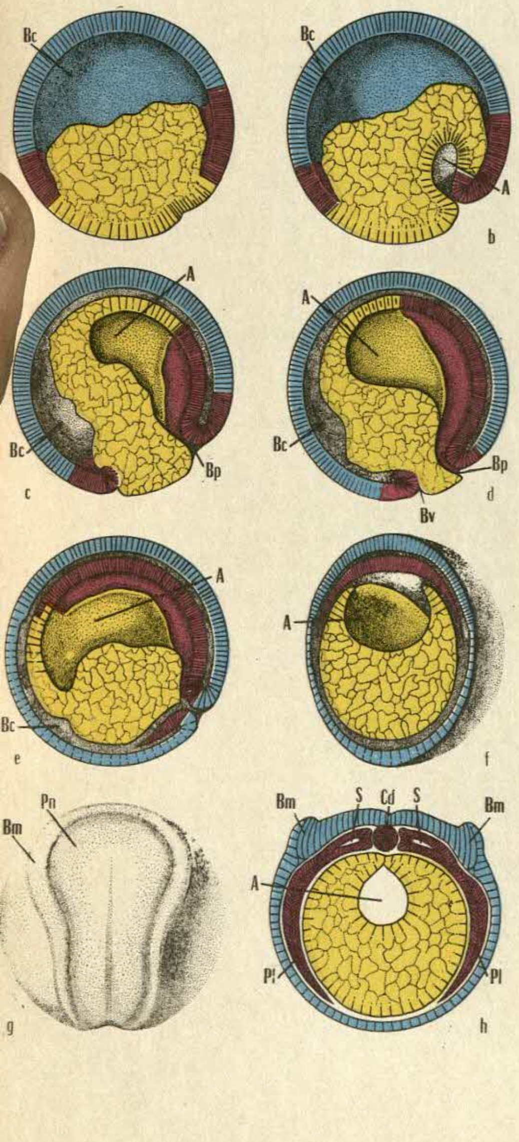

Fig. 3. Diagram of the normal development from blastula to neurula. a, b, c, d, e: successive stages of gastrulation—sagittal section; f: cross-section at end of gastrulation (same stage as e; g: neurula seen from the back; h: cross-section of the neurula. Ectoderm in blue, endoderm in yellow; mesoderm in red. A: archenteron; Bc: blastocoele; Bp: blastopore; Bv: yolk plug; Pn: neural plate; Bm: medullary fold; S: somites; Cd: spinal chord; Pl: lateral plate.

When are the tissues determined?

Once this fact had become clear, new questions arose; *when* and *how* does the determination[5] of the fate of the tissues occur?

The answer to the question of 'when' was explained at the beginning of this century by the German embryologist, Hans Spemann, who carried out grafting experiments, or transplantations, between amphibian embryos at different stages of development.[6] He cut away a piece of one gastrula, and grafted it on to another in a different position, noting its development under the new circumstances.

Spemann carried out transplantations at various stages of development. When, for example, a piece of presumptive neural plate was removed from an early gastrula, and grafted on to the belly side of a new 'host' of the same age, the graft developed in the same way as did its new surroundings, and not in accordance with the region whence it came.

When the experiment was carried out between two late gastrula stages, the result was the opposite. The graft developed in its new surroundings, on the belly, into formations of the central nervous system such as the brain, eyes, and so on. Here, the determination had taken place in the original position, and the piece of the presumptive neural plate could not do otherwise than differentiate itself in accordance with its origin.[8]

These results made it clear that it was during the gastrulation stage that the neural plate had been first given its directions and had then lost its ability to develop in any other direction but into nervous structures. Its development was possible in one way only.

This provided the answer to the question of 'when' the determination occurs. But the other question, that of 'how', proved to be much more complex and difficult to solve.

It is true that Spemann was simultaneously able to trace something of the mechanism which guides the development of certain organs. For instance, mention may be made of the correlation of the development of the eye and the lens which he discovered: the cup of the eye, which comes into being as a projection of the brain, is in very close proximity to the ectoderm which forms the lens. But if the ectoderm which normally develops into the lens is removed, and replaced by a piece of ectoderm from another part of the embryo, this foreign ectoderm is also capable of forming a lens. If the eye were removed from its original position, and grafted under the ectoderm in another part of the embryo, then the lens also would be formed there; yet if some foreign tissue is grafted between the eye and the presumptive lens-ectoderm, the lens would not form.

It became obvious that the development of the eye is guided by a system with two components: the eye is the active component, later known as the action system or the inductor, which determines the development of the ectoderm; the ectoderm is the reacting system obeying the orders of the inductor. These two systems form the induction system.

At that time, nobody had any idea of the way in which the eye could determine the differentiation of the lens: it was simply assumed that the eye provided some mechanical irritation to the ectoderm which made it differentiate. As at this stage of development there are plenty of dividing cells within the eye, it was also supposed that the determination was brought about by a mysterious radiation. A third possibility was that the determinative action was chemical in nature.

The organiser

In the spring of 1921, in Spemann's laboratory, one of his pupils, Hilde Mangold, was engaged in carrying out grafting experiments with a view to defining the exact time of determination of different parts of the early gastrula. She transplanted a piece from the grey crescent into the belly side of another embryo of the same age. The result was very striking indeed; for a second neural plate was formed on the belly of the host which developed into a neural groove, and later into a neural tube. On either side, there were to be seen the ear vesicles and the two rows of somites. The notochord and the finned tail also took shape. A secondary embryo had come into being on the belly of the host.

The second embryo was at first assumed to have resulted from the self-differentiation[7] of a presumptive neural plate determined at an exceptionally early stage.

The experiment was repeated with the embryos of two different species of amphibia, one of which had very pale eggs, and the other much darker ones, it could be seen that only a very small part of the secondary neural plate had formed from the graft, the main part of it having originated in the tissues of the host which had differentiated in directions other than the normal ones.

Meanwhile, Vogt had shown in his experiments that the grey crescent does not develop into the neural plate, but forms the mantle of the mesoderm whose middle part lies under the presumptive neural plate. It was thus impossible that the secondary neural plate had been formed from the graft.

Subsequently, precise experiments were to show that the middle part of the grey crescent, that part from which at the neurula stage the notochord and somites develop, has a very special significance in determination. It is an 'organiser' or an 'organisation centre'.

When the organiser was discovered, it was also obvious that it linked the earlier-known induction systems whose importance lay only in the development of different organs, with a main system which guides the whole development and differentiation of the embryo. The primary organiser directly determines the differentiation of the neural plate, but at the conclusion of this stage of development, secondary organisers are formed in different parts of the neural plate, and these are identical with the action systems whose presence was known previously. Directly or indirectly, different parts of the central nervous system give rise to the development of eyes, nasal pits, ears, gills, teeth, and so on. The main system acts as a chain-reaction in which each step of the development is the result of an earlier occurrence, and the prerequisite for the following ones. This chain-reaction is initiated by the action of the blastoporal lip—by the middle part of the grey crescent, the organiser.

If the whole of the organiser were grafted on to the belly of an early gastrula it would create a complete back of an embryo and, in successful experiments, such secondary formations may be very similar to the back of the host. If the blastoporal lip is removed from an early gastrula, no neural plate can be formed.

Some time afterwards, it was found that if the organiser is bisected along the middle line, each half can act as a whole organiser.

When the organiser is divided horizontally, the part of it which is close to the margin of the blastoporal lip of the early gastrula, and the part above it, have qualitatively different properties in the course of development. The region at the margin, that is, the presumptive forepart of the archenteron roof, tends to induce only head structures, whereas the presumptive rear region can above all induce trunk and tail structures.

The nature of inductive action

In 1932 Spemann stated that even organisers that have been killed are equally capable of inducing secondary formations if they are brought into contact with the competent ectoderm.[8] The organiser may be killed by one means or another, by crushing, by freezing, or by heating, by treatment with different kinds of solvents, alcohol, ether, chloroform, and so on. But nevertheless, it is able to create a neural plate and all kinds of derivatives. The inductive action could no longer be regarded as mechanical irritation by the inductor, nor was it caused by mitotic radiation. It had to be chemical in nature.

As the results of Spemann's school had hinted that the nature of the inductive action might be chemical, integrated teams were formed, in which embryologists collaborated with biochemists, in order to determine the active agency, the 'evocator', as it was called. A great deal of work was done, but the results obtained were very contradictory.

Today it is possible to understand why the teams failed to attain their objective. The background of the whole problem was not clear. It was then supposed that the inductive action was caused by one substance alone. The small size of the eggs brought about many difficulties and when the tissues of the embryos were treated in various solvents, the fractions were always scanty for any adequate examination by ordinary biochemical methods. In addition, we know today that some inductive principles are thermolabile,[9] that is to say, a moderately high temperature inactivates them. The writer, who has been investigating the induction problem for some twenty-five years, has to admit that we still know very little. We know rather more about the mechanism, and of how the organiser acts. We have learnt that in adult tissues, as in the embryonic organiser, there are properties for inducing a neural plate. There are chemical agents which have inducing properties similar to those of Spemann's 'head organiser', and others which behave in a similar fashion to the 'trunk organiser'. We have some idea of their biochemical properties, but so far do not know the exact substances involved. But today we have new methods for these investigations. We have 'tracer' substances, and immunological methods which give us the possibility of revealing minute amounts of substances which are present in the cells. Improved methods are available for cultivation of tissue *in vitro*. There are new methods in biochemistry, paper-chromatography, high-power centrifuges, spectrophotometers, and so on, all of which can analyse substances even when they are present in very small quantities.

By such means, we can at last advance towards the solution of this problem.

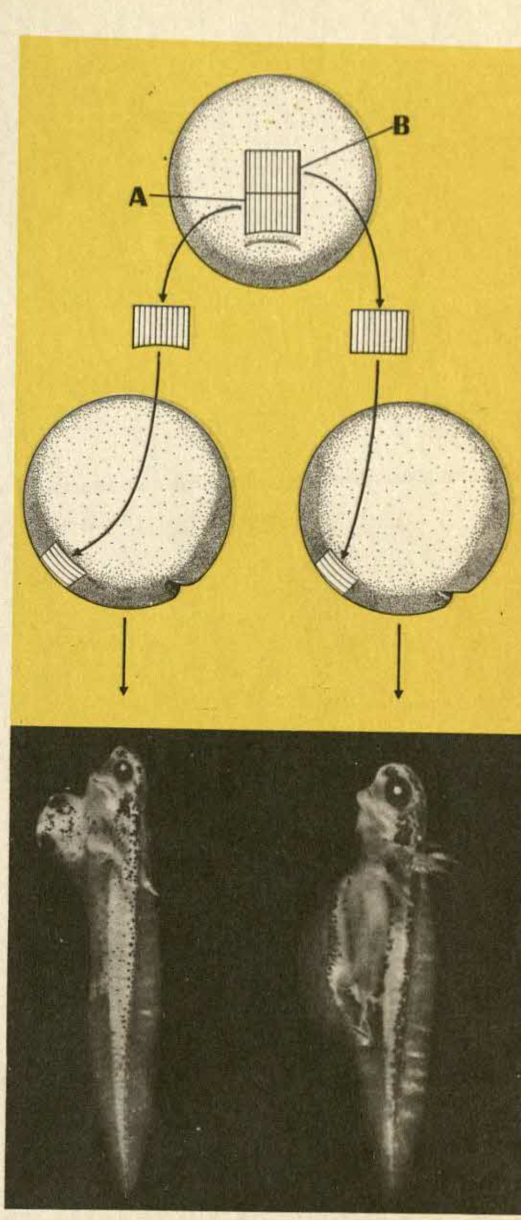

Fig. 4. Diagram of an experiment showing the various inductive properties of two parts of the organiser. If portion A *of the organiser is grafted in the ventral region of another embryo (left), this graft produces a secondary head on the ventral side of the larva. Fragment* B, *on the other hand, produces the formation of an extra body and tail (right).*

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A succession of inductions: the formation of an organ

Etienne WOLFF and Madeleine KIENY

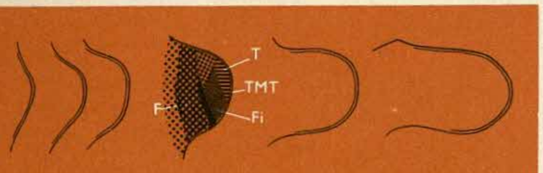


Fig. 1. Development of the leg bud in the embryo of a chicken up to the fourth day of incubation (after Hamburger and Hamilton), with presumptive regions marked on one of the diagrams as follows: **F** region of the femur; **T** of the tibia; **Fi** of the fibula; **TMT** of the tarso-metatarsals.

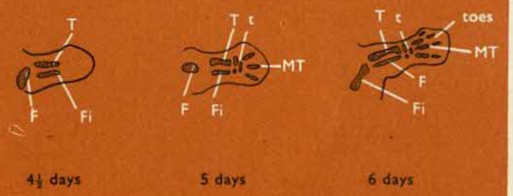


Fig. 2. Differentiation of the skeletal parts of the leg. After 4½ days' incubation, the skeletal elements of the thigh and the leg have become cartilaginous. At 5 days the bones of the heel and metatarsus can be seen. At 6 days the separation of the toes begins. **F** femur; **T** tibia; **Fi** fibula; **t** tarsus; **MT** metatarsus. (After Hampé).

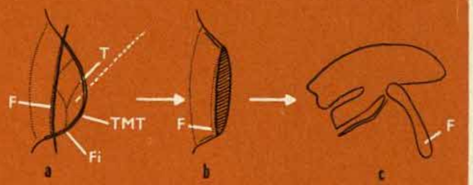


Fig. 3. Excision of the apical crest of a leg bud at 3 days. (a) Amputation of the terminal part composed of the presumptive leg and foot regions. (b) Stump of leg bud comprising the potential thigh region only. (c) The leg which has developed is rudimentary, and lacks the skeletal elements of the presumptive regions which have been excised. (After Hampé).

The term organ implies that the body is composed of different parts, each of which is a morphological or functional unit, or both together. Of course, these parts are not independent of one another but make contributions, which are usually indispensable, to the rest of the organism. However, an organ is a clearly defined entity which is often enclosed in a special covering and sometimes only communicates with other organs by way of the circulation or special ducts. Some organs are formed in the body cavities: these are the viscera. Others develop peripherally and ensure contact with the external world, such as the sense organs and limbs. All have a well-defined form and varying structural complexity.

How are such discrete structures formed and separated from each other? In order to understand this it is necessary to refer back to the stage where the embryo as a whole is still undifferentiated and starts to build up its principal systems. Among the vertebrates these are the endodermal, mesodermal, ectodermal and neural layers. All the organs are derived from these four germ layers.

Some organs have a relatively simple structure to begin with and originate from a single layer. Thus the liver and thyroid arise from endodermal rudiments and, whatever their future complexity may be, their specialised tissues are descended from these initial rudiments.

Other organs have a more complex constitution right from the start, taking their origin from two or more rudiments belonging to different embryonic layers: the limbs, the whole of the skin and its derivatives (hair, feathers, nails) (fig. 4), teeth, lungs and kidneys are formed in this way from two components. The eye and the pituitary gland, too, are developed through the collaboration and interaction of several layers.

Every organ raises the problem as to why it should develop at a specific place and during a particular time. Among the organs of mixed origin, there is also the question of whether one of the layers initiates its development by induction and, if this is so, do the other components exert a reciprocal action on the first? We shall show that recent experiments have given partial answers to these questions.

Development of the limb buds

During the third day of incubation of a chick embryo, two little swellings appear on the lateral body wall on each side. These are the buds of the wing and leg which are situated at the levels of the fourteenth to the twentieth and the twenty-fifth to the thirty-first somites, respectively. From the time it appears the tip of the limb is covered by a characteristic refractive cap, which plays an important morphological role (fig. 1).

If the development of the leg is followed, it is seen that during the fourth day of incubation, the bud enlarges and then turns backwards. This is an indication of the hip joint. On the fifth day the joints of the knee and heel are each marked by a constriction. The future foot is paddle-shaped and the position of the toes are seen as protuberances. The limb elongates and the toes differentiate still further, but they remain joined together by an interdigital membrane. In a duck embryo this membrane will develop into the web of the foot, but in a chicken it slowly disappears and after seven and a half days' incubation it has been reabsorbed, so that at this stage the limb pattern corresponds to that of an adult.

Skeletal differentiation begins at the base of the bud and spreads towards the tip (fig. 2). The femur appears first and is followed by the differentiation of the tibia and fibula, the tarsal bones and lastly the four toes. At first, the skeleton is represented by a mesenchymatous model in which the cartilaginous parts differentiate. Then the cartilaginous skeleton grows, takes shape and finally ossifies.

The fate of the different regions of the limbs may be followed by using the technique of labelling with carbon. This method consists of introducing several particles of carbon under the cap which covers the limb bud. These particles will be found in the pelvis, or in one or other of the three limb joints, according to the stage at which they were implanted. In this way Saunders (1948) has constructed a map of the presumptive regions of the wing and Hampé (1957) one for those of the leg (fig. 1). This method also makes it possible to ascertain that these are fundamental regions which are predetermined very early.

The role of the epidermis

The cap which covers the tip of the bud is made up of a thickening of the epidermis and is called the apical crest. If the development of the bud is interfered with by micro-surgery and the distal third amputated, thereby removing the apical crest, a rudimentary limb develops made up of only the parts of the skeleton whose presumptive regions were left in place after the operation (fig. 3). Thus, the limb bud is unable to undergo its distal differentiation when deprived of its apical crest. But what happens if an epidermal cap containing an apical crest is replaced on the cut surface of a limb stump?

This experiment was carried out in the following way by Hampé (1959): the stump which was to receive the graft was prepared by cutting off a leg bud at the presumptive foot region. An apical epidermal cap, previously removed from another leg bud and separated from the mesoderm by the action of trypsin, was grafted onto the stump. Under these conditions, the distal growth of the bud was resumed and it developed into a leg ending in toes. Without the apical cap, the stump would not differentiate beyond the heel.

An apical cap may also be grafted onto a stump where the leg has been removed at the level of the future knee. In this case the limb bud resumes growth and always differentiates into phalanges, often metatarsals and sometimes a tibia (fig. 10).

If the apical cap of a wing is grafted onto a leg stump, it forms a typical leg with toes. The experiment is still more striking when the apical cap from a limb bud of a chicken is joined to the stump of a leg bud of a duck. The leg which then develops has webbed toes. In this way a duck's leg is formed by joining together tissues from two different species.

The role of the mesoderm

Thus, the growth and differentiation of the terminal parts of the limb are determined by the presence of an apical cap, but the parts of the skeleton which develop always conform to the nature of the mesoderm with which the apical ectodermal cap is in contact.

The important role played by the ectoderm in the differentiation and growth of a limb has been shown, but which of the two limb components, epidermal and mesodermal, is responsible for the early outgrowth of the bud from the side of the embryo?

Grafting experiments performed by Madeleine Kieny have provided evidence for the primary inductive role of the mesoderm. After fifty-five to sixty hours' incubation, the mesoderm is removed from the limb region before the bud has appeared. The fragment is carefully cleared of its ectodermal covering by the action of a dissociating agent, versene. It is then inserted under the ectoderm in the future side region of another embryo, at equal distances from the wing and leg region. This graft results in the formation of a supernumerary limb situated between the wing and normal leg of the host. This extra limb is either a wing, if the implanted mesoderm comes from a presumptive wing region, or a leg, if the graft is presumptive leg mesoderm (figs. 6 and 7).

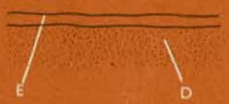
The development of the supernumerary limb takes place in the same way as that of a normal limb. A few hours after implantation, a protuberance forms at the site of the graft which grows rapidly and soon assumes the appearance of a normal limb bud. Under the inductive action of the graft, the covering ectoderm differentiates into an apical cap. This possesses all the properties of a normal epidermal crest. In fact, if it is regrafted onto the stump of a normal leg bud, which has been cut off at the level of the future knee, this cap brings about the resumption of the distal growth in the operated limb (fig. 8). All this takes place in the same way as if the grafted apical cap had been taken from a normal leg.

The experiments that have been described enable us to interpret the mechanism of limb formation in the following way: at a very early stage (twenty somites), the lateral mesoderm induces the ectoderm to thicken and form an apical crest. Then this ectodermal crest acts in turn on the mesoderm and induces the growth and differentiation of the limb. The nature of this differentiation (whether wing or leg, chicken or duck) is always determined by the nature of the mesoderm of the limb bud.

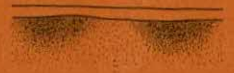
Determination and regulation

Thus, the fate of the limb seems to be clearly predetermined both as a whole and in detail. The future joints may be demarcated precisely: which part will form the femur, and

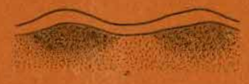
Fig. 4. Differentiation of the skin and feather buds in a chicken embryo (diagrammatic sections). The skin of birds is formed of two parts: the epidermis, of ectodermic origin, and the cutis, of mesodermic origin. The feather starts as an excrescence on the skin of the embryo, called the feather bud (in the same way scales develop from scale buds).



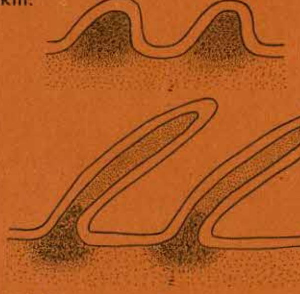
(a) Sixth day of incubation. The skin is not differentiated. The epidermis, **E** is flat, the dermis, **D** is homogeneous.



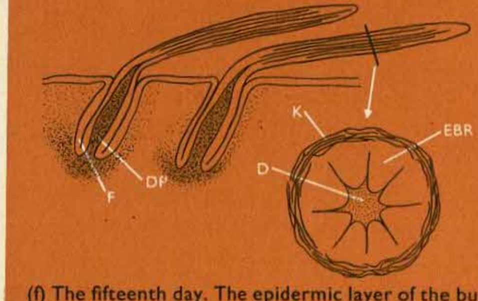
(b) The seventh day. The cells of the dermis condense in some places. They form little lumps, the feather rudiments, at regular intervals under the surface of the epidermis—which is still flat and undifferentiated.



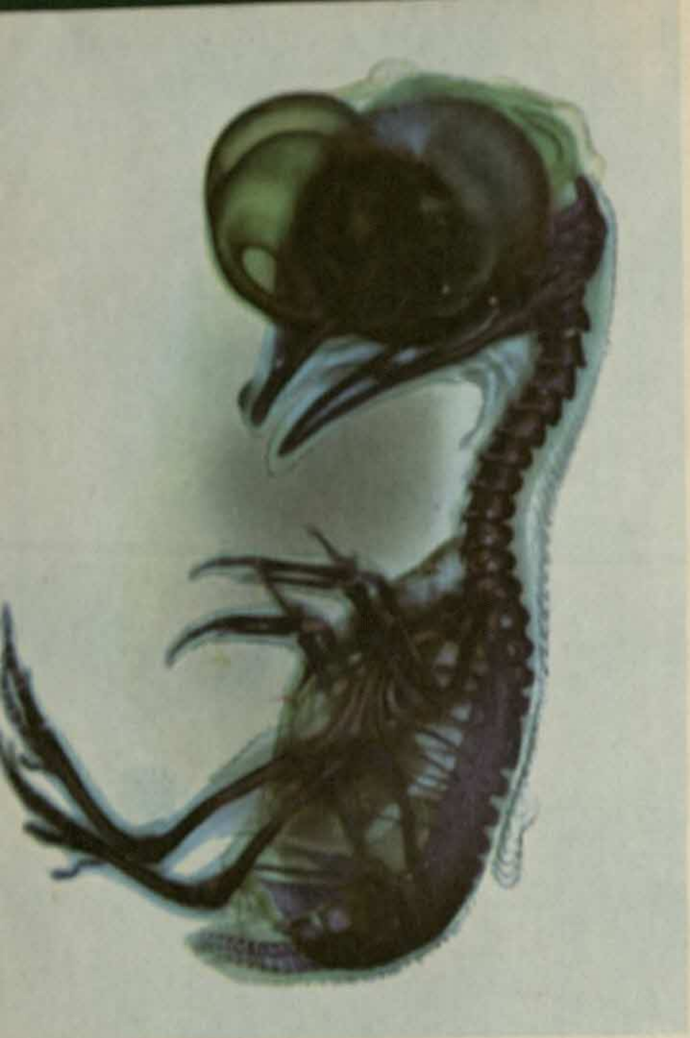
(c) Under the inductive action of each feather bud, the epidermis differentiates in its turn, thickens, and then puts out little papillae on the surface of the skin.



(d and e) From the eighth to the tenth day. These papillae, the so-called feather buds, lengthen considerably. At this stage the epidermis reacts on the dermis and induces the dermic cells to colonise the epidermic layer. The epidermis is also responsible for the direction of growth of the bud, directing its free extremity towards the tail of the embryo.



(f) The fifteenth day. The epidermic layer of the bud has made a second differentiation. Longitudinal ridges are raised which give rise to barbs and barbules. The external layer of the epidermis becomes keratinised, forming a horny sheath which will be shed after the opening up of the feather on hatching. At the base of the feather bud, the epidermis is embedded in the dermic mesenchyme to form the permanent follicle of the future feather. The nature of the dermis determines the quality of the epidermic differentiation. The dermis of the body induces feather buds, the dermis of the feet induces scales, **EBR** epidermic barb ridge; **D** dermis; **E** epidermis; **F** follicle of the future feather; **K** superficial layer turned to keratin; **DP** dermal papilla of the future feather.



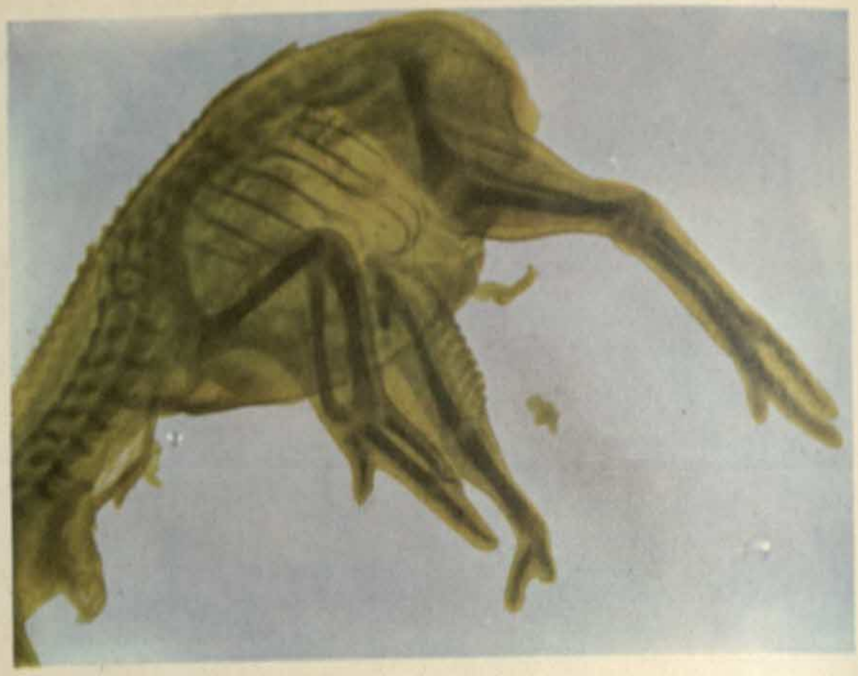


Fig. 5. A ten-day-old chicken embryo whose skeleton has been shown up by selective colouration of the cartilage (toluidine blue). The general arrangement of the skeletal elements corresponds to that of the adult. (Photo Lod).

*Fig. 6. Inductive role of the mesoderm. The mesoderm of a future leg bud from a twenty-six somite embryo has been grafted into the side of a nineteen somite embryo. It has developed as a harmonious*ly *formed supernumerary limb between the wing and the leg of the host. The femur, tibia and two toes can be made out. (The fibula is lacking). (Photo Lod).*

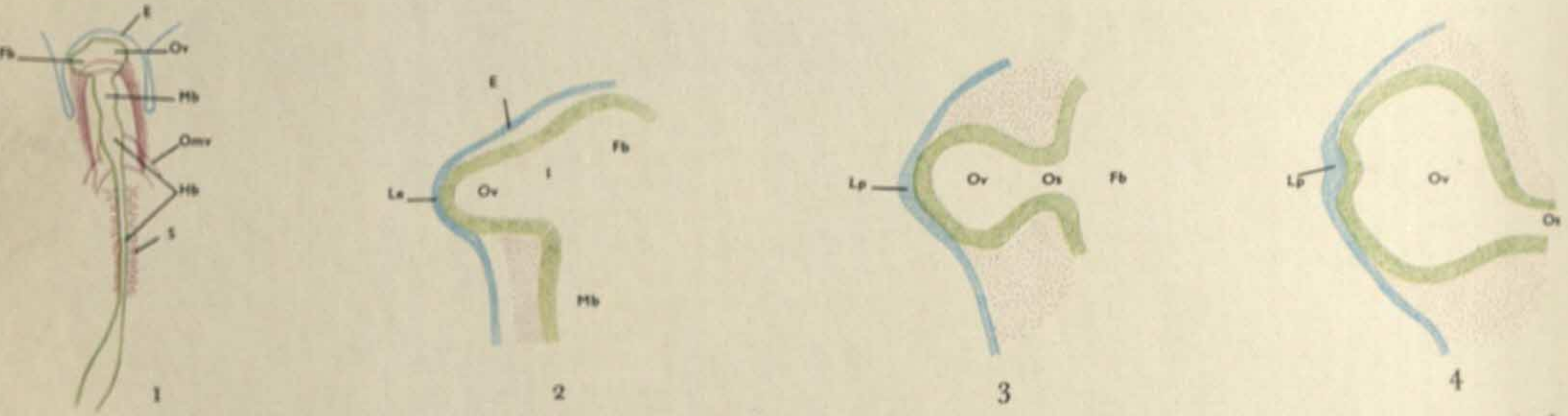


Fig. 9. The eye is an organ which is built up from neural tissue from the brain (green), head epiaermis (blue), and head mesoderm (red).

1. Embryo after thirty-five hours' incubation: the brain comprises three parts: fore, middle and hind brain. The largest part of the forebrain has dilated into two lateral expansions, which are the first rudiments of the eyes, known as optic vesicles.

2. At forty-two hours: the optic vesicles have moved away from the brain by becoming constricted at the base. The optic stalk will form the future optic nerve, and the vesicle itself the future eyeball. The lateral wall of the optic vesicle has come into contact with the lateral epidermis.

3. At fifty hours: under the influence of the induction of the optic vesicle, the epidermis has thickened to form the lens placode, the first rudiment of the crystalline lens. The optic stalk has narrowed.

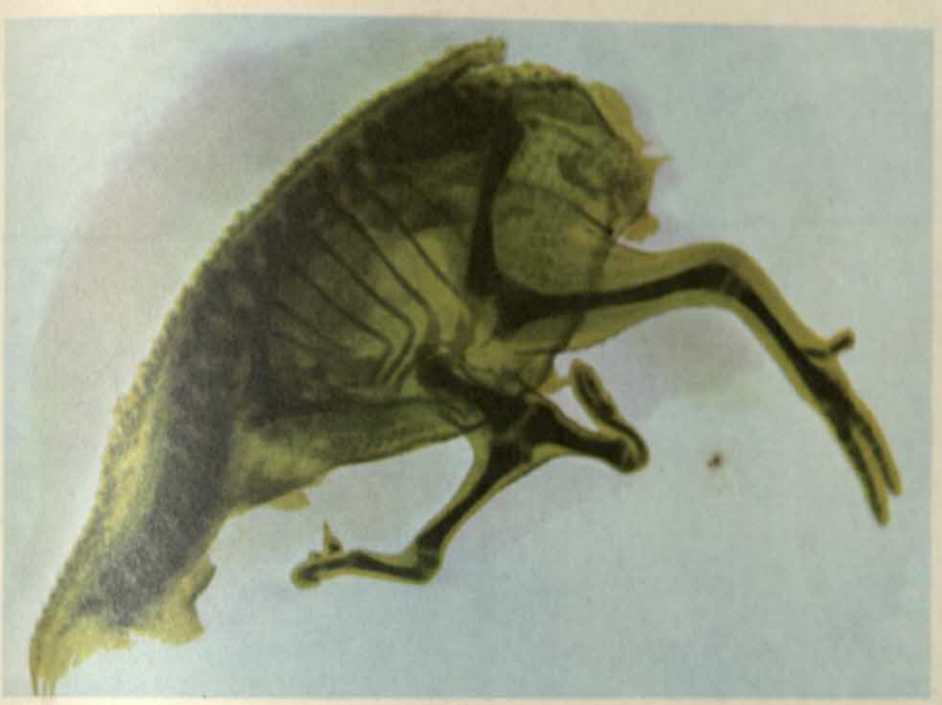
4. At fifty-two hours: the optic vesicle has begun to invaginate. The lens follows this invagination and remains in contact with the lateral wall of the vesicle.

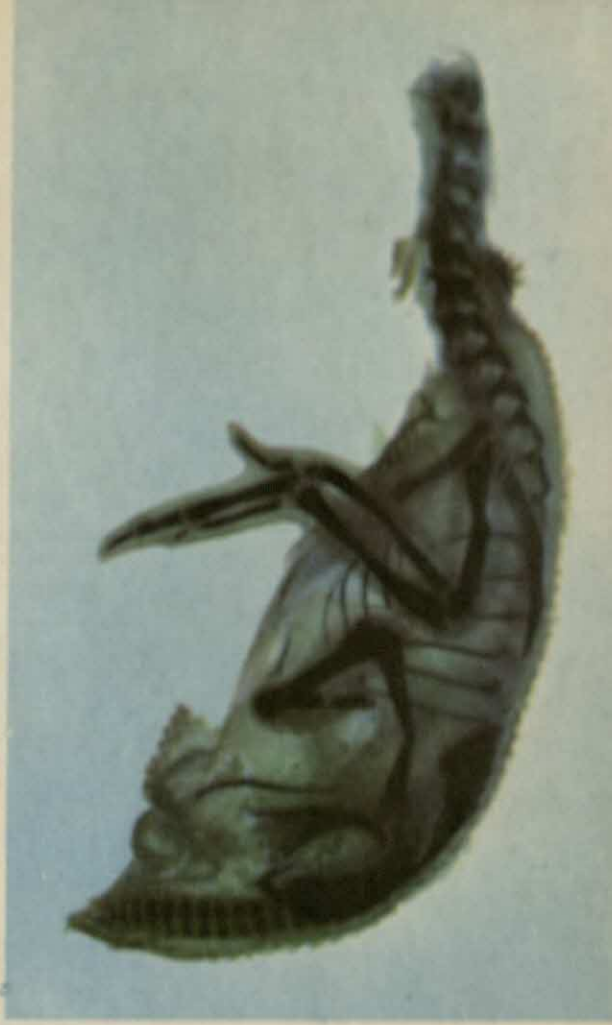
5. At fifty-five hours: the placode has been transformed into a lens vesicle which is still open to the exterior.

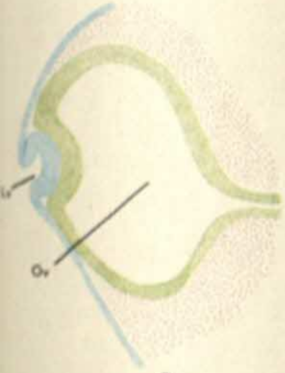
6. At three days: the crystalline vesicle has closed up on itself and has detached from the epidermis, whose surface has rejoined. The invagination of

Fig. 7. *The inductive role of the mesoderm. The same experiment as the one in Fig. 6, but here the supernumerary limb has divided into two parts: there is a common femur, and two tibias each ending in two toes. (Photo Lod).*

Fig. 8. *The typical morphological role of the apical crest of the limb bud of a supernumerary limb, which has been grafted onto the stump of the leg bud of another embryo. (The plan of the operation is the same as that shown in Fig. 10.) The leg has developed almost normally. It is made up of a femur, a tibia and a toe with two phalanges. (Photo Lod).*







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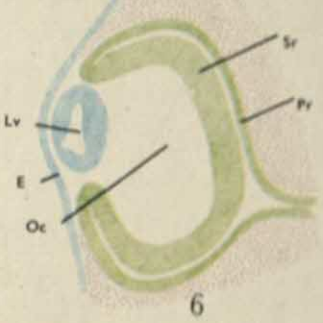

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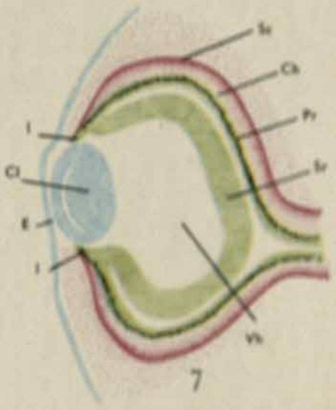

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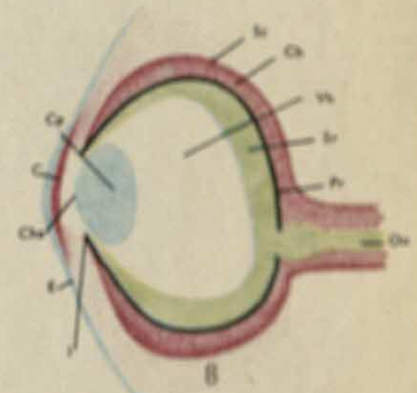

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the lateral wall of the optic vesicle continues until the cavity is completely obliterated. In this way the optic cup is made up of a double wall: the thin external wall, gives rise to the pigmented layer of the retina; the thick internal wall to the sensory retina.

7. At four days: the lens has formed. At this stage the mesodermal cells surround the eyeball and differentiate into the nourishing and protective layers of the eye, the choroid and sclerotic. The edge of the optic cup in the neighbourhood of the lens, together with the mesoderm which infiltrates at this level collaborate to form the iris. The cells of the external layer of the optic cup become filled with black pigment.

8. At 7–8 days: under the inductive action of the optic cup other mesodermal cells invade the growing space enclosed between the lens and the epidermis. These participate with the epidermis in creating the transparent cornea. Between the cornea and the edges of the iris (pupil) the anterior chamber of the eye is hollowed out, while the eyeball behind the crystalline lens fills with a gelatinous fluid, the vitreous humour.

Cl: *crystalline lens*, Fb: *fore brain*, Ch: *choroid*, Cha: *anterior chamber of the eye*, Mb: *mid brain*, Oc: *optic cup*, Hb: *hind brain*, Vh: *vitreous humour*, He: *head epidermis*, Le: *lens epidermis*, I: *iris*, C: *cornea*, On: *optic nerve*, Lp: *lens placode*, Os: *optic stalk*, Pr: *pigmented layer of retina*, Sr: *sensory layer of retina*, S: *somite*, Sc: *sclerotic*, Lv: *lens vesicle*, Ov: *optic vesicle*, Omv: *omphalomesenteric vein*, E: *epidermis*.

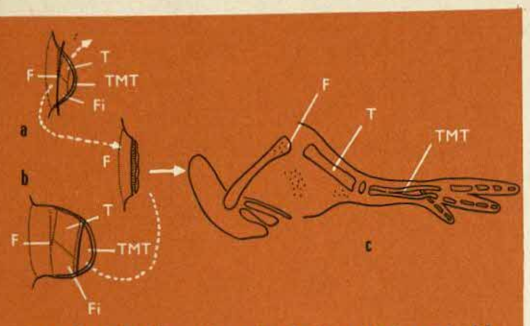


Fig. 10. Morphogenic role of the apical crest. (a) Amputation at three days of the presumptive regions of the leg and foot. (b) The surface of the section of the stump is covered by an ectodermic apical crest cut from a leg bud of another embryo. (c) The leg developed is almost normal. It lacks only the fibula and great toe (compare fig. 3). (After Hampé).

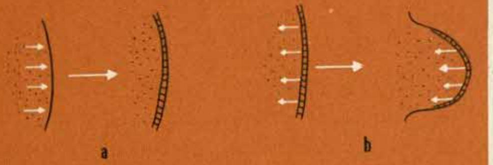


Fig. 11. Diagram of successive inductions which lead to the formation of the limb bud. (a) The mesoderm provokes thickening of the ectoderm below it: it induces the formation of the ectodermic apical ridge. (b) This ridge in turn reacts on the mesoderm and determines its growth and differentiation from the base to the extremity.

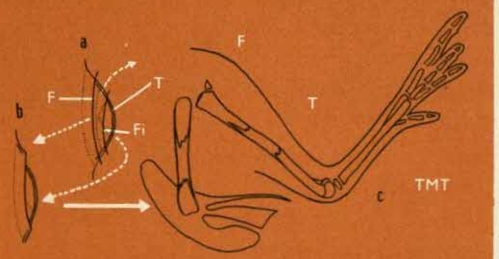


Fig. 12. Rectification of deficiency. (a) Excision by two transverse sections of a middle segment corresponding to the future tibia and fibula. (b) After removal of the middle segment, the apical crest is grafted on to the base stump. (c) Despite the missing material, the developed leg is well proportioned; the fibula is missing. **F** femur; **T** tibia; **Fi** fibula; **TMT** tarso-metatarsals. (After Hampé).

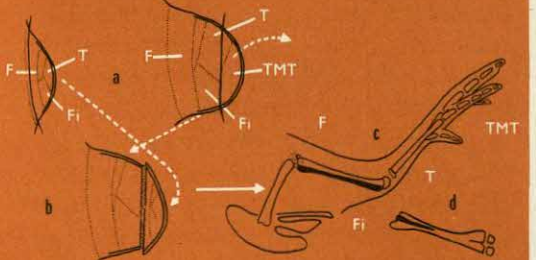


Fig. 13. Rectification of excess. (a) Amputation of the region corresponding to the future tarso-metatarsals from a leg bud of 3½ days. (b) An entire leg bud of three days is grafted on the stump. (c) In spite of the excess material, the leg develops harmoniously. The fibula (in black) is abnormally long, its distal extremity reaches as far as the tarso-metatarsal joints. (d) In a normal leg the fibula hardly exceeds half the length of the tibia. (After Hampé).

which the tibia, alongside which the fibula develops shortly afterwards. These structures are not visible but the potentiality to develop into them is there and their form is predetermined. At this stage, the limb bud apparently consists of a mosaic of parts. A coloured particle, inserted into one of these parts, will be recovered where it would be expected to turn up, according to the shape mapped in theory; also a local burn will destroy exactly that part of an organ, or region of the body, corresponding to the potentiality ascribed to it. For example, if the region of an embryo, which is a presumptive tibia, is grafted on to another part of the embryo, it develops into a tibia.

However, this development is not final. Living matter has resources which enable it to repair serious damage. The leg bud of a three-day old chicken embryo is made up of approximately three zones corresponding to thigh, leg and foot. Supposing we cut out the segment which should give rise to the lower leg, and attach the terminal cap, corresponding to the potential foot region, onto the stump of the thigh (fig. 12). One of us, engaged in this experiment, was surprised to notice a few days later that the mutilated bud had given rise to a limb of normal appearance. The lower leg was present and well formed. It cannot be said to have been restored, because it had never previously existed, but the two remaining parts had collaborated to build the missing segment. This property, which is often manifested by embryonic rudiments, is termed regulation. It enables the organism to repair losses during a period when it is still labile. (This power of regulation of parts of the limb bud was first shown experimentally by P. D. F. Murray in 1926.)

Many variations on this experiment have been carried out by Hampé. We shall mention only one, which is to some extent the opposite of that just described. Instead of taking away a rudiment from a bud, an attempt may be made to graft extra material into it. In this way a slice, comprising a prospective thigh and leg, may be taken from one embryo and grafted in between the cap, corresponding to the foot, and the rest of the limb bud of another embryo (fig. 13). The resulting limb might be expected to show a succession of parts arranged in the following way: thigh-leg-thigh-leg-foot, with their constituent bony parts. But when the graft 'takes' under good conditions, the results are quite different. Instead of a large disorganised leg, the one that forms is normal in shape and size and has the usual sequence of segments. The extra material has been integrated and both the supplementary parts and the normal potential embryonic regions have fused into a harmonious whole. This property is not peculiar to a limb bud. In this particular case the result shows that, although the embryonic shapes are apparently predetermined, they are still plastic and capable of important modifications.

However, although these legs are normal in appearance and have apparently not been upset by such drastic operations, they should be examined more closely. This may be done by using the appropriate treatment to render them transparent, thereby showing up their skeleton with greater intensity and relief than by x-ray.

If the limb that has succeeded in restoring its missing region is examined, it will be found that it has a tibia of normal shape and length, but no fibula (fig. 12). In contemporary birds this slender little bone ends in a tapered point towards the middle of the tibia (fig. 13d); it is the non-functional rudiment of the well-developed fibula which is found among the majority of vertebrates and in the ancestor of the birds, *Archaeopteryx*. Thus, when there is a serious loss of material, the tibia is able to form, but this vestigial part tends to disappear.

If the limb which has succeeded in integrating a large excess of material is examined, it is found that the harmoniously developed leg contains the usual bony parts: femur, tibia, fibula, tarso-metatarsals and phalanges. Nothing is abnormal, either in number or structure, except for the fibula, which is a complete bone of the same length as the tibia, and which, like the tibia, articulates with the tarsals (fig. 13). Thus this experiment may be said to have restored the ancestral type of fibula. Therefore, according to whether there is a lack or excess of material, the fibula disappears completely or reappears in its primitive form. The tibia has priority of development and takes advantage of all the available material, whilst the fibula makes use of the remainder, or surplus if any.

Nothing serves better than these experiments to emphasise that the fibula of birds is an atrophied bone which is at present undergoing regression. It is, in fact, disappearing and by appropriate intervention it is possible to evoke its primitive condition, or to foretell its fate. Such researches lead us towards a new study, which might well be called experimental palaeontology.

The pictures are of the experiments by Madeleine Kieny at the embryology laboratory of the Collège de France and the C.N.R.S.

The development of the vertebrate sex organs

Albert RAYNAUD

All prospective parents wonder whether their child will be a boy or a girl, but few suspect the complexity of the processes which combine to produce the sexual characteristics of an individual, whether male or female.

In this article I intend to summarise the major stages in the development of the sex organs, especially as they occur in mammals. These steps form a chain of successive reactions, starting with the fertilised egg. Numerous investigators have been working to elucidate these processes for more than a century and their early studies enabled us to follow the morphological sequence of sexual development. Then, with the introduction of experimental methods over the last thirty years or so, valuable information has been gained by the use of sex hormones and various techniques for interfering with the embryo's development.

The material on which these researches have been carried out is as varied as the tints of an artist's palette: the richly-pigmented embryos of fish and amphibians; the young bird embryo resting on its yolk, surrounded by a pink halo of vascular tissue; lizard and snake embryos encased in their parchment-like shells, and finally, mammalian embryos, concealed within the body, bathed in amniotic fluid,[1] and doubly protected by the foetal membranes and the maternal uterus. It is now possible to experiment on all of these and many of their secrets have been revealed.

However, to return to the original problem: how are the male and female genital organs formed, and what factors control their development? In order to understand their formation it is necessary first to consider the egg when it has just been fertilised. The hereditary factors (genes) for sex[2] provided by the sperm and egg give the fertilised egg either a male or female genetic constitution. Therefore, sex, that is genetic sex, is determined at the moment of fertilisation (fig. 2), but masculinising and feminising factors are present in both sexes and it is the predominance of one or other of these that determines the sex of the fertilised egg and directs all the later sexual development of the embryo.

New data were provided when the sex chromosomes[3] were discovered among invertebrates and some groups of vertebrates. Thus, among mammals, the cells of males have two special chromosomes which are distinct from the other chromosomes, known as autosomes. These are designated by the letters X and Y. On the other hand, the cells of females have two X chromosomes. These chromosomes separate during meiosis[4] and each passes to a different gamete.[5] In this class of vertebrates the genetic factors for femaleness are carried both by the X chromosomes and the autosomes at the same time. The genes for maleness are located chiefly on the Y chromosome.

Recent experiments have provided further information. There are certain mice which

MAJOR STAGES IN EMBRYONIC SEXUAL DEVELOPMENT

sex genes
↓
primary morphogenetic substances
↓
sexual differentiation of gonads
↓
embryonic sex hormones
↓
sexual differentiation of the genital tract

One theory (E. Witschi's) regards the primary morphogenetic substances as distinct from the sex hormones. Another (E. Wolff's) regards the primary morphogenetic substances as the same as the sex hormones of the embryo and the adult.

(1) Amniotic fluid: *the fluid enclosed by the coverings surrounding the embryo. It provides an aquatic environment which, by bathing the embryo, protects it from shocks and prevents distortion, since the fluid exerts the same pressure over the whole surface of the embryo.*

(2) Sex genes: *genes are complex molecules which are rich in nucleic acids and are arranged along the chromosomes. These molecules possess the property of self-duplication and are the determinants of hereditary characteristics. The particular set of genes belonging to an individual is termed the 'geneone'; this controls the constitutional characteristics of 'genotype'. The sex genes determine whether the gonads of the embryo will develop into testes or ovaries.*

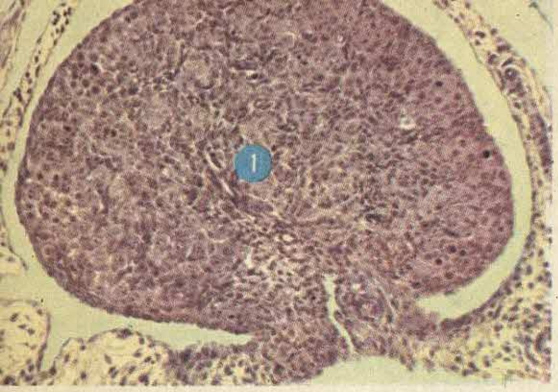
Fig. 1. *Sexual differentiation of the genital organs of mammals*

(1) *The young embryo is sexually undifferentiated: the gonads on the median edge of the mesonephros are sexually undetermined and a double set of genital ducts is present—the potentially male Wolffian ducts and the potentially female Müllerian ducts. The urogenital sinus and the external genitalia are also undifferentiated.*

(2) *When the embryo is a genetic female the genital system develops as follows: the gonads become ovaries, the Wolffian ducts regress, only the tips remaining at each end. The Müllerian ducts develop and differentiate into funnels, Fallopian tubes, uterine horns and the body of the uterus. The urogenital sinus is partitioned into a ventral urethra and a vagina, or into a utero-vaginal passage.*

(3) *When the embryo is a genetic male, the genital system differentiates as follows: the gonads become testes and descend towards the scrotum. The Müllerian ducts degenerate (only the top and bottom ends remain, the latter forming the uterus masculinus). The Wolffian ducts become the* vas deferens, *which gives rise to the buds of the seminal vesicles. The urogenital sinus becomes the urogenital duct and gives rise to the buds of the prostate glands and Cowper's glands. The external genitalia are transformed into a rudimentary penis. (Diagrams by Albert Raynaud).*

The four photographs on the left of this diagram represent the sex organs of newly born mice in histological section. (a) female mouse, showing ovary (1) (mag. × 143); (b) female mouse: transverse section of the urogenital sinus in the process of being partitioned into a dorsal vaginal rudiment (2) and a ventral urethra (3) (mag. × 106); (c) male mouse: seminal tubules (4), interstitial cells (5), testis (6), Wolffian duct (7), (mag. × 58); (d) male mouse, showing male urethra (3) (mag. × 73). (Text by Albert Raynaud, photos Sengel).



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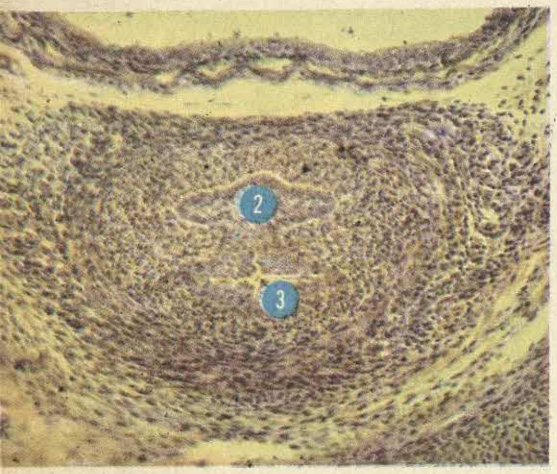

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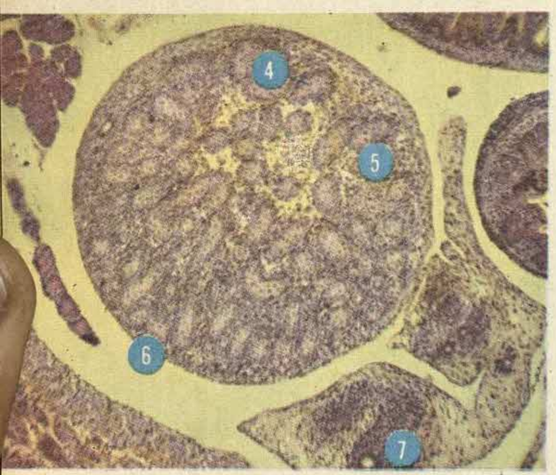

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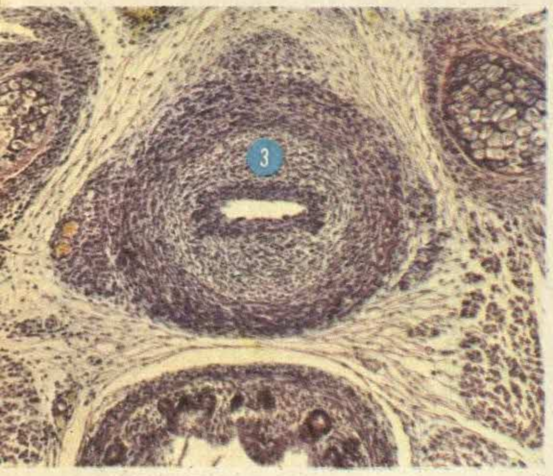

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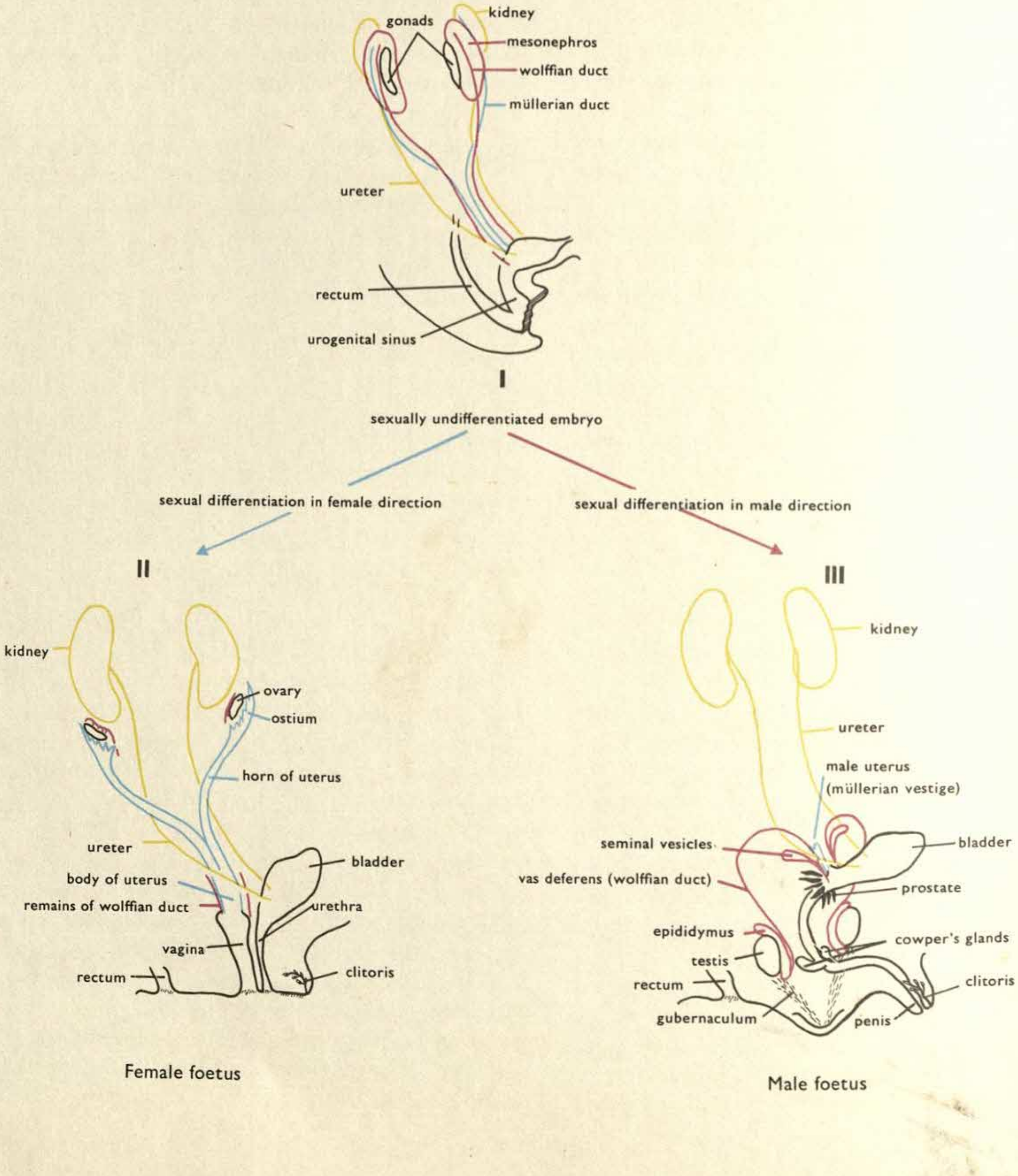


possess only thirty-nine chromosomes instead of the normal forty. These mice are female and the missing chromosome is an X chromosome; their constitution is therefore 2A + XO (A = autosomes).

The comparison of the three formulae:
2A + XX: normal female with forty chromosomes
2A + XO: normal female with thirty-nine chromosomes
2A + XY: normal male
leads us to conclude that the Y chromosome carries the male sex determinants. The factors for femaleness are situated mostly on the autosomes, although some of them are probably on the X chromosomes.

Sex determination in the human species has recently been assumed to take place by a mechanism similar to the one just described for mice. This hypothesis follows from recent studies of abnormalities which probably result from the failure of the sex chromosomes to separate at the time of gamete formation. By combining the study of the chromosome assortment and 'nuclear sex'[6] it has been shown that some individuals have an extra chromosome. Their diploid number[7] is forty-seven instead of forty-six and they have the constitution 2A + XXY. These individuals are male. Given that the formula 2A + XX is that of a woman, and 2A + XY that of a normal male, it may be deduced that in men, as in mice, the Y chromosome is the male sex determinant. These XXY men are sexually abnormal, being sterile with small testes (the germinal epithelium and Sertoli[8] cells are missing and seminal tubes are hyaline,[9] but the interstitial glands[10] are well developed—Klinefelter, Reifenstein, Allbright syndrome). The opposite condition also occurs and these individuals have female morphology, forty-five chromosomes, which are 'sex chromatin negative' and the constitution 2A + XO. They are also sexually anomalous (gonadial aplasia[11] or Turner's syndrome). The genetic analysis of these abnormalities is being actively pursued.

From genetic sex determination to the development of the embryonic gonads

The sex of an egg is determined at fertilisation, as we have seen, but how do the genital organs of an embryo develop in accordance with its genetic sex? This occurs in successive stages. The early development of the gonads is the same in all embryos, but subsequently the organism progressively acquires its sexual characters by the action of certain chemical substances on its immature tissues. These substances channel the development in either a male or a female direction.

Some time after the beginning of the embryo's development the rudiments of the gonad appear along the median edge of the mesonephros[12] on both sides at the base of the mesentery.[13] At first the rudimentary gonads are structurally similar in both sexes. A superficial thickened region, the cortex, formed from the coelomic epithelium,[15] covers the medulla, which is composed of a mass of epithelial cells of extra-gonad origin. This is the undifferentiated stage in the morphogenesis of the gonads.[14] Then, at a later stage, strands and tubules develop in the epithelial nucleus of the male gonad which are the rudiments of the seminiferous tubules.

At first the rudiments of the external genitalia are alike in both sexes. Then the rudiment develops into a penis in the male foetus and into the clitoris in the female.

Thus the embryo is undifferentiated for a period before it acquires its sexual characteristics. This differentiation takes place from the head towards the tail. First the gonads develop, then the ducts, and finally the external genitalia. The chronology of this development for a human embryo is indicated in the table on page 146.

The physiological mechanism of sexual differentiation

We must now consider the factors that bring about this development of the embryonic genital system in such a complicated and remarkably accurate way. It has been shown that the fertilised egg is male or female according to its genetic constitution and, in consequence, all the embryonic cells derived from the division of this egg will inherit the same constitution.

These sex genes become active and cause the cells of the undifferentiated gonad to synthesize chemical substances. These are the primary morphogenetic substances which cause the undifferentiated gonad to develop

(3) Chromosomes: *these are the threads of chromatin (nucleoprotein) found in the nucleus. They are stained strongly by basic dyes at the time of cell division and their form and number are constant for a particular species. The genes are located on them.*

(4) Meiosis: *special mode of cell division which ensures that the number of chromosomes is reduced by half (reduction division).*

(5) Gametes: *reproductive cells. Spermatozoa are produced by the male, ova by the female.*

(6) Nuclear sex: *in 1949 a small body of chromatin was*

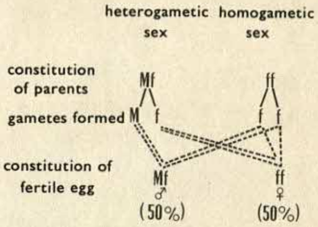


Fig. 2. Hereditary sex determination. One of the sexes (heterogametic) produces two sorts of gametes with respect to sexual make-up (M = male determinant, F = female determinant). The other sex (homogametic) produces gametes, which are all alike with respect to their sex genes. The various combinations of gametes produced at fertilisation give a constant sex ratio (50 per cent males and 50 per cent females). This diagram is, however, too simple. Some workers stress that sex determination is effected by a couple of genes. Therefore M *represents the sum of several masculinising factors and* F *the sum of feminising factors. But in each sex, on the other hand, the potentialities of the opposite sex are present, so it is more accurate to represent the male sex by the formulae* MM Ff *and the female sex by* MM FF; M, F, f, *being of unequal strength.* FF>MM>Ff.

discovered in the nucleolus of a cat's nerve cell. This 'nucleolar satellite' is absent or rare in males, but present in all females. It is also present in other tissues, especially the epidermis. In females it forms a convex mass of chromatin, termed 'sex chromatin' joined on to the nuclear membrane. It represents a fused portion of the two X-chromosomes and thus demonstrates their presence in any individual in a most accurate way, enabling 'nuclear sex' to be defined.

(7) Diploid number: *the normal chromosome number of an individual '2n'. This number is halved to 'n' at gamete formation and restored to '2n' at fertilisation.*

(8) Sertoli cells: *somatic cells in the walls of the seminiferous tubules, between which the spermatozoa develop from the germ cells. These cells maintain and nourish the germ cells.*

(9) Hyalinisation: *degenerative transformation of the cells and components of connective tissue.*

(10) Interstitial gland: *the collection of secretory cells situated between the seminal tubules. This is 'Leydig's gland' and has been shown to be the source of the male secretion of the testis.*

(11) Gonadial aplasia: *abnormal formation of the rudimentary gonads, shown by a lack or extreme reduction of these organs.*

(12) Mesonephros: *an embryonic kidney which has a temporary existence in mammals.*

(13) Intestinal mesentery: *a membranous fold of coelomic epithelium supporting the intestine.*

(14) Gonad: *a genital gland; it has been described as an 'incubator' of germ cells.*

(15) Coelomic epithelium: *the epithelium lining the body cavity.*

(16) Cowper's glands: *accessory glands of the male genital system, formed from two buds arising from the distal part of the ureter wall.*

(17) Gonoducts: *embryonic genital ducts (Wolffian and Müllerian ducts).*

TABLE

Sequence of development of sex organs in the human embryo

First appearance of gonadial rudiment (genital crest) in four-week-old embryo (4-5 mm).

Differentiation of testes in 6-7 week-old embryos (15-25 mm): formation of sex cords (future seminal tubules) in epithelial nucleus of the gonad: formation of tunica albuginea around testis.

Differentiation of ovaries: any gonads which have not differentiated seminal tubules in the epithelial nucleus are destined to form ovaries during seventh week of development: by the end of the second month ovarian cortex is well differentiated.

Genital ducts: Wolffian duct has already formed (from mesonephric duct) in the 4th-5th week. First rudiment (ostium tubae) of Müllerian ducts appears in the embryo when it has reached a length of 10 mm. By the time it is 28 mm long, these ducts have extended to the urogenital sinus.

During the second month of foetal life the two types of duct coexist in embryos, male or female.

In the male embryo, the Wolffian duct develops; the seminal vesicle is budded off during the thirteenth week; Müllerian ducts start to regress in the 27 mm embryo.

In the female embryo, Müllerian ducts continue to develop; Wolffian ducts start to degenerate in the 30 mm embryo.

The paths of development of the external genitalia in the different sexes start to diverge at the tenth week. In the three-month embryo these organs have distinctive characters of their sex.

into either a testis or an ovary. The differentiated glands in their turn secrete secondary morphogenetic substances, or embryonic hormones, which direct the further development of the undifferentiated genital ducts. Thus, the foetal testis produces a masculinising substance which stimulates the development of the Wolffian duct, inhibits the Müllerian duct,[17] gives rise to the development of the accessory glands (seminal vesicle,[18] prostate and Cowper's glands),[16] and causes the external genitalia to develop in the male manner.

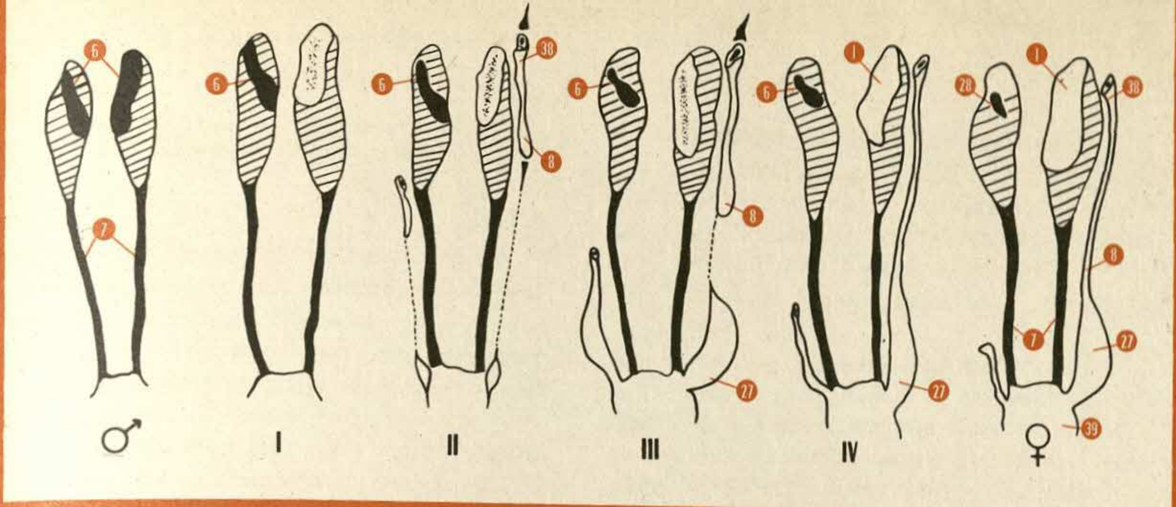
The experimental study of sexual differentiation

The key to the problem is given by the foregoing account, which summarises the essential results of experimental research. However, lest the apparent simplicity of these conclusions should prove misleading, it must be emphasised that detailed research and years of work have been necessary in order to obtain these data. Nevertheless, each step surmounted has thrown new light on the subject.

It is immediately apparent that there is still a great deal of uncertainty about the detailed processes underlying the phenomena of sexual differentiation of the gonads. However, some accurate results have been obtained; for it is possible to modify the course of development of an undifferentiated gonad by parabiosis[19] between amphibian larvae of different sexes, or by the grafting of gonads, or the use of sex hormones. There are various interpretations of these results, but it is impossible at this stage to tell which one corresponds to the processes actually taking place in the normal differentiation of a gonad.

There are two main alternative theories. First, the hypothesis of cortical and medullary inductors, elaborated by Professor E. Witchi, and based on the assumption that the cortex and medulla[20] of the undifferentiated gonad produce substances which act as inductors.[21] Thus, the substances produced by the follicle cells of the cortex would have a feminising effect and provoke the development of the ovarian cortex and cause the undifferentiated germinal cells[22] to become oocytes. On the other hand, the medulla would produce a masculinising substance which would cause the gonad to develop into a testis. There is an antagonism between these two systems of inductors so that, since the male sex genes predominate in the male, the medulla dominates the cortex and a testis develops, while the reverse takes place in the female embryo. These embryonic inductor substances would be different from the sex hormones and be released only during a very restricted period of embryonic development.

The second hypothesis is that held by Professor Etienne Wolff which suggests that all sexual differentiation may be considered as due to one and the same category of hormones acting throughout the whole development of the individual, from the beginning of organogenesis to the adult stage. The sex genes themselves would produce the hormones[23] which orientate the histogenesis of the gonad towards the testicular or ovarian type. In a young male embryo, this hormone production will take place in the cells of the interstitial tissue of the undifferentiated gonad. This theory is chiefly supported by the fact that adult sex hormones are capable of reversing the histogenesis of the embryonic gonads in the same way as embryonic gonadal grafts do in the bird embryo.



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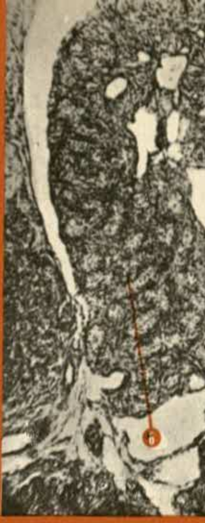

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Fig. 3. The action of sex hormones on the differentiation of a chicken embryo. Diagram of the genital systems of twenty-day-old chicken embryos. On the left, a normal male embryo after twenty days' incubation: the testes (6) and Wolffian ducts (7) are shown in black. On the right, a normal female embryo (eighteen days' incubation): left ovary (1), right atrophied gonad (28). The right oviduct is rudimentary, the left (8) is well developed, with its funnel (38), its shell gland (27) and cloaca (39). I, II, III, IV: *intersexual male embryos obtained by the action of oestrogen. In* I *the effect of the hormone is shown on the left gonad, which has been transformed into an ovotestis. In* II *some Müllerian segments have also persisted. In* III *the left gonad has an ovarian structure and the right testis is reduced. The Müllerian duct is almost complete, with its funnel (38) and shell gland (27). In* IV *the genital system of this male intersex strongly resembles that of a normal female: there is a left ovary and a right testis. The left oviduct is continuous, the right reduced to a posterior segment. (After E. Wolff and A. Ginglinger, 1935, E. Wolff, 1946).*

Fig. 4. Action of sex hormones on the sexual differentiation of a chicken embryo. Histological section of the left gonad of a male intersex embryo. The ovarian cortex is well developed around a nucleus of reduced testis (6). (35: cortex, 34: lacunae). (Photographs by Professor Etienne Wolff).

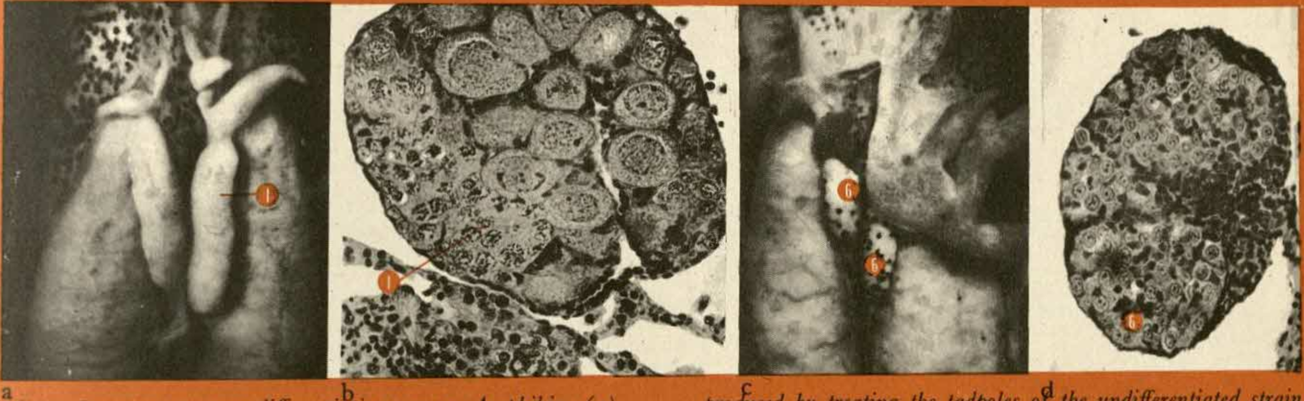

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Fig. 5. Action of sex hormones on sex differentiation among Amphibia. (a) external appearance of ovary; (b) histological section of ovary. (a) and (b) ovary of frog tadpole (Rans temporaria) *belonging to an undifferentiated strain. In this strain all the individuals have female genital morphology at metamorphosis and among them there are 50 per cent males and 50 per cent genetic females. (c) external appearance and (d) histological section of the pigmented testes (6) produced by treating the tadpoles of the undifferentiated strain of* Rana temporaria *with a male hormone (testosterone propionate). As a result of this treatment, all the individuals have a male genital morphology at metamorphosis and 50 per cent of these individuals are masculinised genetic females. The masculinisation of the latter is permanent. (Photographs by Professor L. Gallien, who carried out the experiments).*

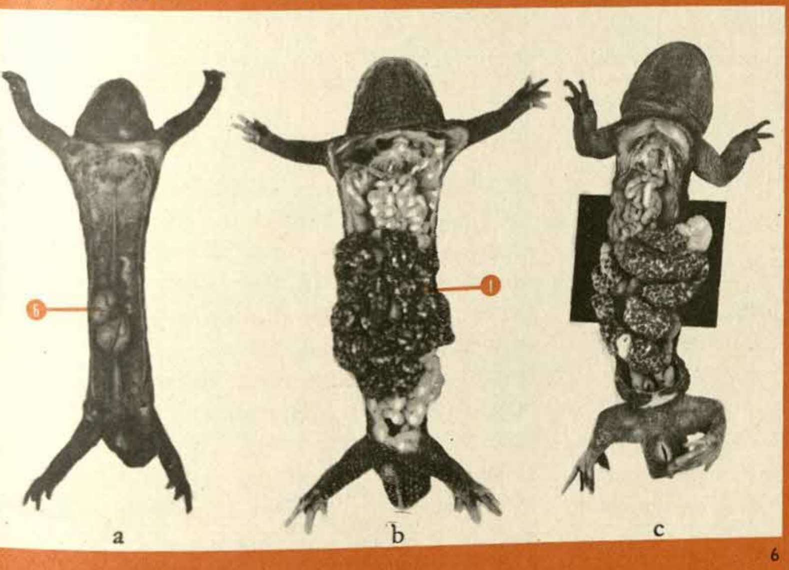

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Fig. 6. Action of sex hormones on sex differentiation in amphibians: (a) and (b) Pleurodeles (Pleurodeles watlii M.); *normal adult individuals of male sex (a) (testis, 6) and (b) female (1: ovary); (c)* Pleurodeles *intersex obtained by treating a genetic male with a female hormone (oestradiol benzoate).*

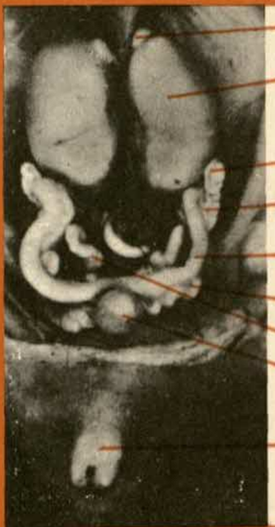

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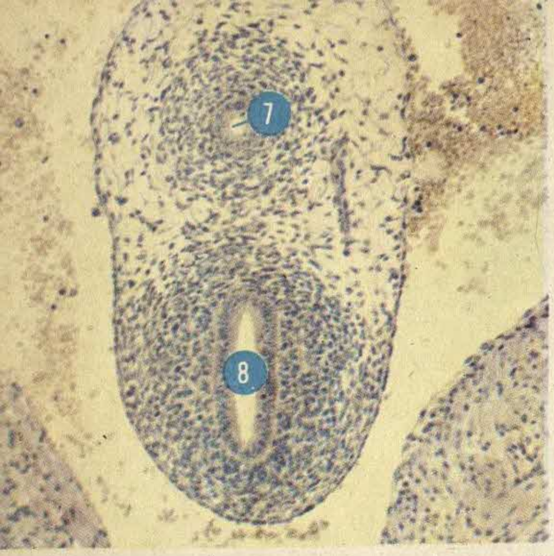
Fig. 7. Genital system of a three-month-old mouse, masculinised by the action of testosterone propionate treated as an embryo and after birth: beside the female organs (1: ovaries, 24: uterine horns) numerous male organs have developed (23: epidydimis, 25: vas deferens, *10: seminal vesicles, 26: male copulatory organ). (21: adrenal capsules, 22: kidney, 15: bladder). (Photo Albert Raynaud).*



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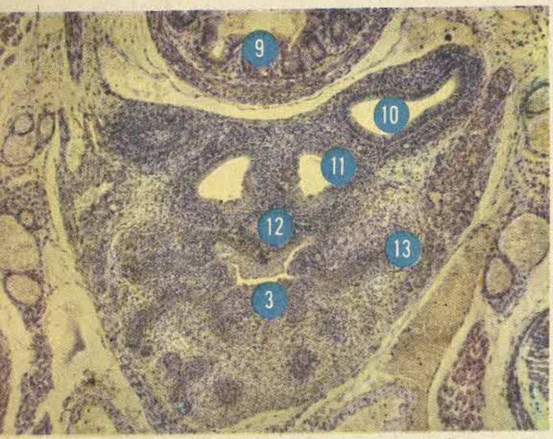

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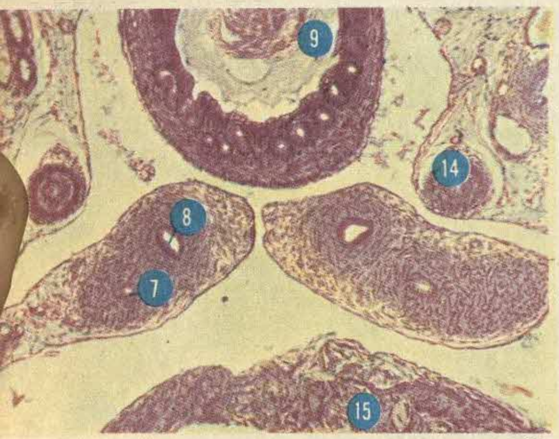

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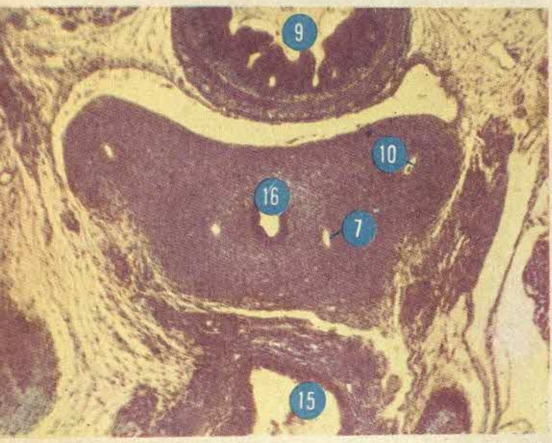

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Meanwhile the question remains as to whether the sex hormones act directly on the rudimentary gonads or simply modify their inductor systems. Many arguments have been put forward, for or against both theories, but no definite conclusion has yet been reached.

Concerning the second stage, we have accurate sources of information drawn from observation and experiment. This is the stage when the sexually differentiated gonads act on the embryonic genital tracts. One of the most spectacular examples is the phenomenon of the 'free-martin' cow.[24] If twins of different sexes are linked by vascular anastomoses while in the cow's uterus, the female twin is modified. Her genital apparatus is masculinised; the ovaries are inhibited and sometimes assume a testicular structure; the Wolffian ducts are stimulated; the Müllerian ducts are inhibited and accessory male glands develop. This transformation is due to the hormones secreted by the male embryo's testis entering the female twin's circulation, where it stimulates the development of the male sexual rudiments of the genital tract[25] which would normally degenerate.

To elucidate the nature of such changes three major types of experiment have been conducted on this stage of the development of the sex organs. These are experiments on parabiosis or embryonic grafts, the study of the effects of sex hormones, and, finally, foetal castration.

Fig. 8. Masculinisation of female embryos by action of a male hormone. Histological sections of the sex organs of a new-born female mouse coming from a mother which has received injections of testosterone propionate during gestation. (8a) Wolffian ducts (7) coexist with Müllerian ducts (8) in each urogenital fold (mag. × 141); (8b) female showing ejaculatory canals (11), male ureter (3) and some prostatic buds (13) (mag. × 57); (12: prostatic utricle, 9: rectum, 10: seminal vesicle). (Text by Albert Raynaud, photo Sengel).

Fig. 9. Feminisation of a male embryo by an oestrogenic hormone. Section of genital ducts of a new-born male mouse from a mother which has received 1mg dihydrostilboestrol (synthetic oestrogenic hormone) during pregnancy: (9a) Wolffian ducts (7) and Müllerian ducts (8) have developed (mag. × 56) (9: rectum, 15: bladder: 14: ureter); (9b) a male mouse showing uterine body (16), as well as vas deferens *(7), and seminal vesicles (10). (15 bladder, 9 rectum) (mag. × 56). (Text by Albert Raynaud, photo Sengel).*

Parabiotic experiments and grafts

When two young amphibian embryos (frog or *Amblystoma*) are grafted on to each other shortly after the closure of the neural tube, they remain joined together and develop side by side. If the embryos are of different sex, it is then noticed that the development of the female embryo is considerably upset; the ovaries being atrophied or masculinised. Sometimes, however, it is the male partner which is feminised. Similar effects have been produced by grafting an embryonic gonad directly into an immature animal.

Several experiments performed on birds have thrown light on the secretory activities of embryonic gonads. The ovary of a young female chicken embryo was carefully introduced into the body cavity of a male embryo. The graft took, became active and secreted and, under its influence, the left gonad of the host was transformed into an ovotestis[26] and the Müllerian ducts were stimulated. Conversely, a testis grafted into a female embryo inhibited the development of the Müllerian ducts.

In the same way, the testis of a young rat embryo grafted into the seminal vesicle of a castrated adult stimulated the epithelium of this gland, thus providing evidence for the secretion of a masculinising substance by the testis.

All these experiments demonstrate that the embryonic gonads secrete substances which are capable of influencing the development of the gonads and genital passages of the embryo.

The effect of hormones on sexual differentiation

The functioning of the embryonic gonads has been demonstrated by the above experiments and observations. However, when the chemical nature of the substance (sex hormones) secreted by the adult gonads became known it was natural to ask whether the embryonic gonads also acted on the undifferentiated genital tract by liberating substances analagous to the adult sex hormones. Experiments to find out if this was the case were carried out as soon as possible and turned out to be highly informative.

The early work consisted of extensive experiments carried out on chicken embryos. The results were spectacular. When a drop of female hormone (oestrogen, oestradiol) was introduced into a young, sexually undifferentiated embryo it produced very marked effects. If the embryo was male, an ovarian cortex developed on the surface of the testis (figs. 3 and 4) and the Müllerian ducts persisted and hypertrophied. Thus the male embryo was feminised and ended up resembling a female embryo completely. When the transformation was less pronounced, the characters that developed were intermediate between both sexes (varying degrees of intersexuality). The male hormones (testosterone group) have a less powerful action and chiefly affect the development of the genital tracts, provoking the degeneration of the Müllerian ducts and the stimulation of the Wolffian ducts.

These experiments had scarcely been completed when the effects of sex hormones on amphibian embryos were tested. Here, too, the results surpassed all expectations. Among the higher Anurans (the frogs and toads, Ranidae and Hylidae) testosterone completely masculinised the gonad. On hatching, all the individuals which had been injected at the stage when the tadpoles were sexually undifferentiated were morphologically male. This masculinisation was permanent and a year after the injections the gonad continued to develop like a testis (figs. 5 and 6). The action of the female hormones was shown to be more complicated; for, in strong doses, they had the paradoxical action of masculinising female embryos, whereas with weaker doses all the tadpoles were feminised and possessed ovarian-type gonads (fig. 6).

These transformations brought about by the sex hormones are so profound that the modified gonads thus 'reversed' are capable of producing gametes. In this way it has been possible to transform genetic males into functional physiological females. When their progeny have been studied after crossing with normal males, they have been found to consist exclusively of individuals of only one sex.

Among mammals the action of the sex hormones on the embryonic development of the gonads has been shown to be just as powerful. The initial experiment consisted of injecting a drop of an oily solution of testosterone propionate into a young female guinea-pig embryo while it was still in the uterus. The hormone brought about masculinisation of the embryo.

Similar results have been obtained by injecting the sex hormone into the mother during gestation, so that it was carried by the circulation and reached the embryo after crossing the placenta. This method was used by workers in the United States on rats and by myself on mice. Thus, after administering doses of 5-10 mg of male hormone (testosterone propionate) to gravid mice on the thirteenth day of gestation, the female foetuses were found to be masculinised. An entire male genital tract developed alongside the ovaries and female genital passages. The Wolffian duct persisted and the male accessory glands (seminal vesicles, prostate and Cowper's glands) developed. The urogenital sinus was transformed into a male ureter and the rudiments of male external genitalia started to differentiate (fig. 8).

These male sexual structures persisted after birth in females masculinised in this way and when these adult females were submitted to fresh injections of male hormones, the complete development of the male genital tract was effected (fig. 7): the accessory glands secreted copiously and the sexual behaviour of the female was also reversed. This effect of the hormone is not restricted to small laboratory rodents, for the foetus of the female monkey (*Macaca rhesus*) has been shown to be markedly masculinised by testosterone.

Conversely, the oestrogenic hormones feminise the male foetus and under their action Müllerian ducts, a uterus, a female ureter and a rudimentary vagina have all been produced in a male mouse foetus.

In these various experiments which have been carried out on placental mammals, the effects of the sex hormones usually show up in

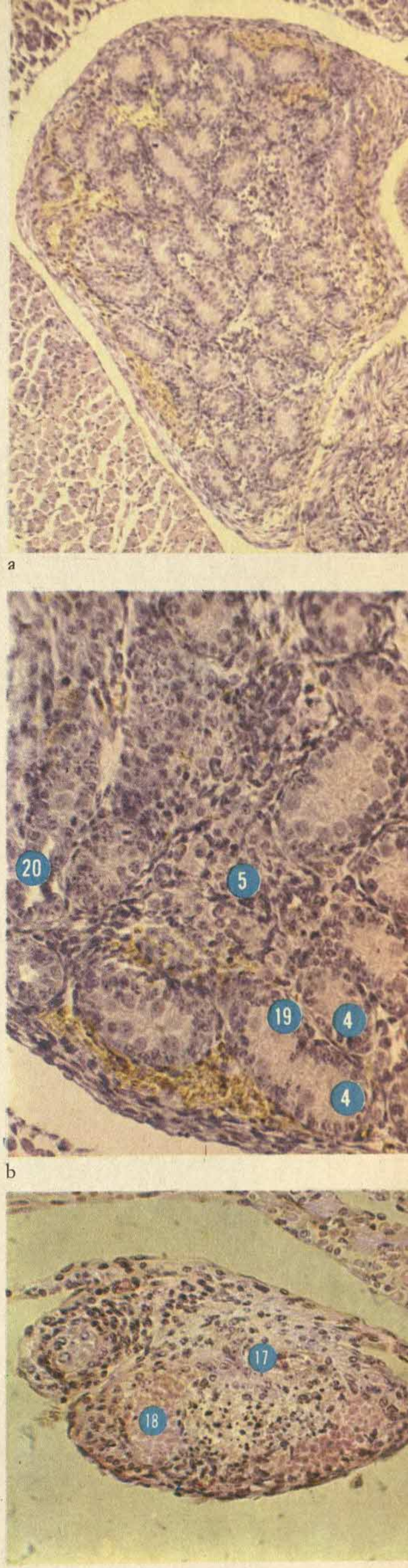


Fig. 10. Destruction of a foetal testis by localised irradiation with X-rays. *(10a) General view of a normal testis from an eighteen-day-old mouse foetus (mag. × 110). (10b) An enlarged portion of testis showing the rete (20), the testicular tubules (4) and a mass of interstitial cells (5). (19: germinal cell) (mag. × 280). (10c) Testis destroyed by* X-rays *(100,000 administered at thirteen days, killed at eighteen days). All the constituents of the testis (stroma and interstitial cells (17) have been killed by radiation by the rays (mag. × 280) (18: Remains of testicular tubules). (Text by Albert Raynaud, photo Sengel).*

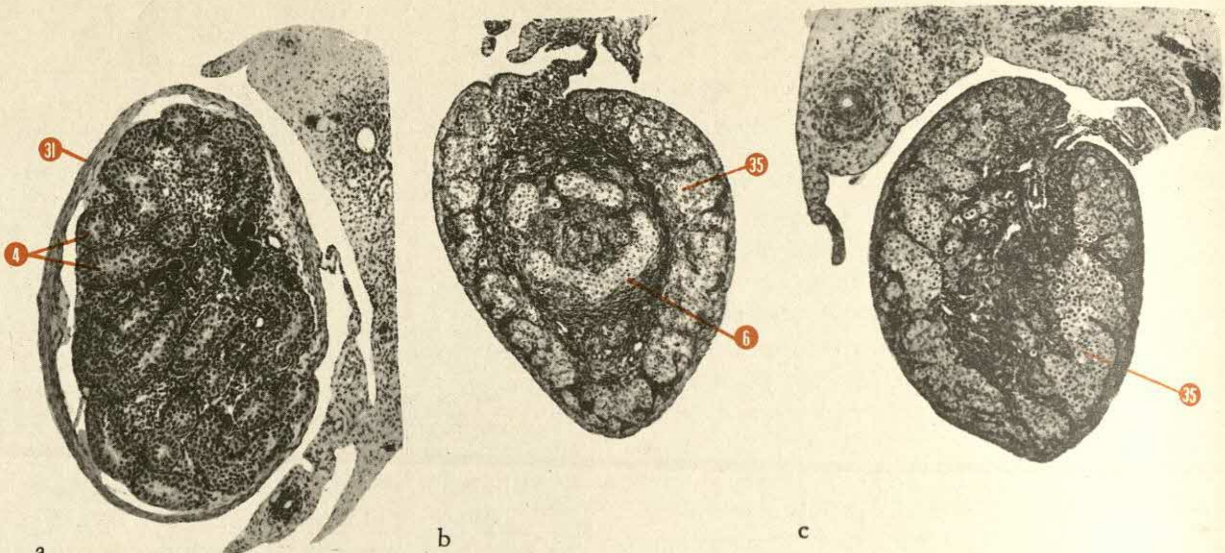


Fig. 11. Section of a gonad from a one-month-old opossum (Didelphys marsupialis)*; (a) testis of a normal male (31: albuginea, 4: seminal tubules); (b) testis transformed into a mixed gland (ovotestis comprising a peripheral part (35) with the characters of an ovarian cortex, enclosing a reduced testicular part (6). This ovotestis was obtained by treating a male opossum daily from birth with an average dose of 0·12 mg of oestradial dipropionate; (c) ovary of a normal female, the cortex (35) is well developed. (Photos by permission of Dr R. K. Burns, Professor of Embryology at Baltimore University, U.S.A.).*

the development of the genital passages (gonoducts and urogenital sinus), but one research worker has succeeded in transforming the testis of a marsupial (opossum—*Didelphys virginiana*) into an ovotestis by using very weak doses of female hormone (fig. 11).

Foetal castration

Since the male sex hormone, testosterone, is capable of stimulating a male genital tract in a female embryo, and the female sex hormones or oestrogens are capable of stimulating the development of a female tract in a male embryo, the experiments described would appear to satisfy the less exacting investigator. For it seems highly probable that the gonads of the normal embryo secrete sexual hormones which control the development of the genital tract. But caution must be exercised, since such inductive reasoning does not satisfy the conscientious experimenter who demands direct proof.

In order to obtain this proof, the functioning of the gonads of the embryo must be demonstrated either by their removal or destruction and the effects of their suppression studied. In other words, it is necessary to carry out foetal castration. This is a difficult operation, since the mammalian foetus is bathed in amniotic fluid and protected both by special membranes and the wall of the maternal uterus. It was only in 1946 that such castration experiments could be satisfactorily performed early enough in development and with a sufficiently long survival of the operated foetuses.

The consequences of removing the gonads surgically from rabbit and mouse foetuses were demonstrated in a series of experiments. With a collaborator, I succeeded in destroying the gonad of an embryo by localised irradiation[27] with x-rays (fig. 10). Finally, in 1946, the surgical castration of a rat was achieved two or three days before the end of gestation.

These experiments reveal two facts: first, after the suppression of the foetal ovaries the development of the sex organs of the female foetus proceeds in an apparently normal way. Is female sexual differentiation therefore autonomous, as was foreseen as early as 1934 (single hormone theory)? It is possible, but still too early to be able to confirm it, for the uterine environment, in which the castrated foetus develops, is not without hormones.

Second, after the ablation or destruction of the testes, the masculinisation of the genital tracts of the male foetus does not take place. The Wolffian ducts atrophy, or remain slender, the accessory glands (prostate, seminal vesicles) do not develop, and the external genitalia do not become masculinised but assume a structure like those of a female. Besides this, the Müllerian ducts do not regress, but remain and differentiate, as in a female, and the urogenital sinus also develops

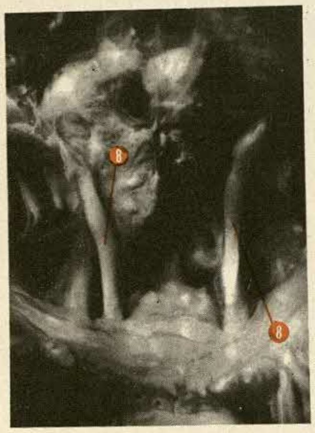
(18) *Seminal vesicles: accessory glands of the male genital system formed by budding from the posterior part of the Wolffian ducts.*

(19) *Parabiosis: uniting two embryos, or two adults, by grafting or a temporary join, so that they then live side by side with circulatory and humoral exchange.*

(20) *Cortex and medulla of the gonad: the two active regions of the rudimentary embryonic genital gland. The cortex is situated at the periphery of the rudiment (derived from coelomic epithelium) and has the potentiality of developing into ovary, while the medulla is the central region and has the potentiality of developing into testis.*

(21) *Inductor substances: those with the property of determining the differentiation of an embryonic rudiment.*

Fig. 12. Persistence of Müllerian ducts (8) in a male embryo after castration (photo E. Wolff, 1950).



In birds, the embryonic testes bring about the degeneration of the Müllerian ducts, but the development of the male genital tubercle and the male syrinx is not hormonally controlled. In the female embryo the ovarian secretion causes the right Müllerian duct to degenerate and feminise the rudiment of the syrinx and the genital tubercle.

in the female way and gives rise to a female ureter and vaginal rudiment. Thus, sexual differentiation after castration is deflected towards femaleness (fig. 13).

Unilateral castrations and the implanting of a crystal of androgenic hormone into a castrated male foetus confirm that the effects of castration are due to the suppression of a hormonal masculinising secretion of the foetal testis. It is thus clear that the male gonad causes the embryonic genital tract to develop in the masculine direction by secreting a male hormone. This hormone has two types of action: it both acts as a stimulator, giving rise to the development of the Wolffian ducts, the accessory male glands, and the genital tubercle, and as an inhibitor, which causes the Müllerian duct to regress and prevents the urogenital sinus becoming partitioned. It is this double action that results in the male foetus's genital system acquiring masculine characteristics.

However, experiments on rabbits have shown that the functioning of the foetal testis is under the control of the foetal pituitary gland.

Most of the physiological processes which link up to control the development of the male or female genital system are now known. This knowledge has enabled us to explain, either genetically or hormonally, abnormalities of sexual development, particularly the masculinisation of females brought about by the action of a male hormone during foetal development; and the feminisation of males, which may result either from the action of an oestrogenic hormone or from the cessation (or alteration) of the functioning of the foetal testis. (To cite an example: the genital organs of individuals with gonadial aplasia are of feminine type. Studies of their nuclear sex has shown that there are some men among these individuals and that their feminine morphology can now be explained as due to a lack of secretion of male hormone during the embryonic stage.) The powerful action of the sex hormones, androgens and oestrogens on the genital system, should prompt the banning of their use during pregnancy.

Many questions remain unanswered, but the analysis of those complex embryonic sexual processes which has already been carried out is an encouraging achievement.

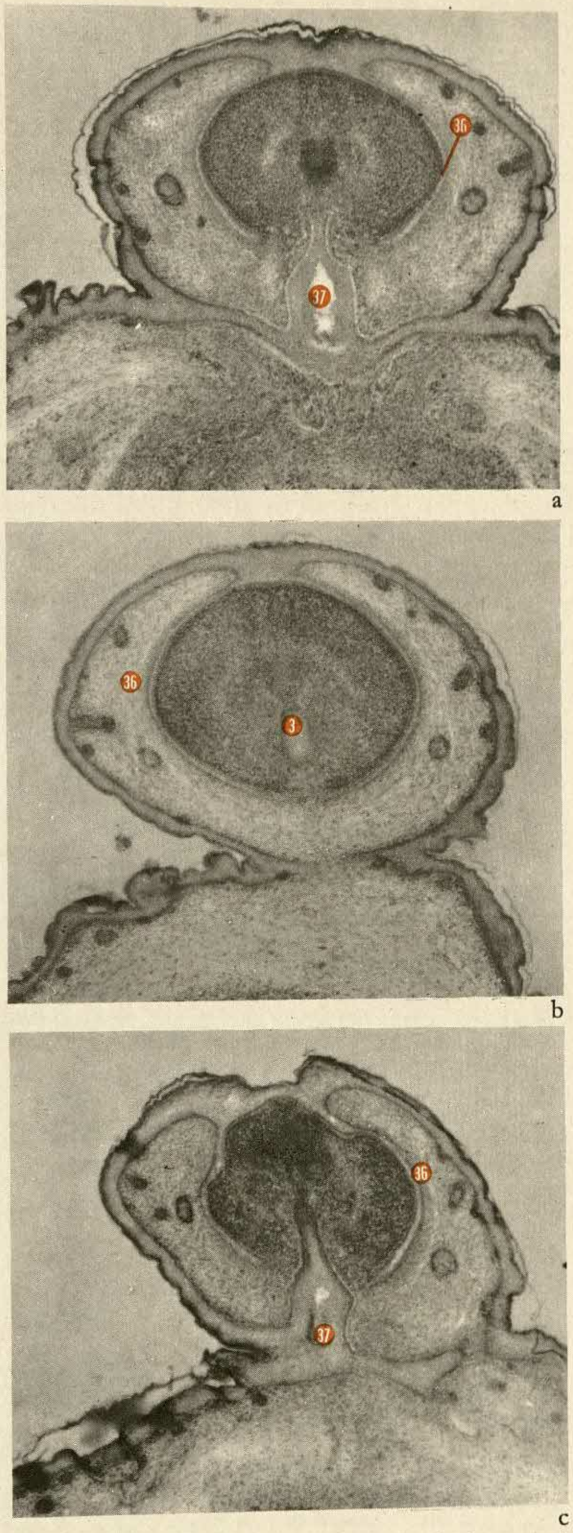


Fig. 13. Effects of foetal castration on the development of the external genitalia in a mouse; (a) section of the genital tubercle of a female foetus aged eighteen days (37: urogenital opening, 36: preputial fold); (b) section of the penis rudiment of a normal male mouse foetus aged eighteen days (36: balano-preputial fold, 3: ureter); (c) section of the genital tubercle of a male mouse foetus aged eighteen days. The testes have been destroyed by localised irradiation with X*-rays at thirteen days. The development of this rudiment has taken place in the female manner (36: preputial fold, 37: urogenital opening).*

(22) Germ cells: *mother-cells of the reproductive cells (oogonia and spermatogonia). These are separated very early on during the segmentation of the egg and comprise a special line of descent within the individual, forming an independent unity, the germ line, besides the other tissues (forming the 'soma').*

(23) Sex hormones: *chemical substances secreted by the genital glands. Their structure is based on derivatives of cholesterol. Cited here are the 'ovarian hormones', of which the chief ones are the* C *18 steroids (the oestrone and oestradiol group), and the hormones of the corpus luteum of the ovary (*C *21 steroids) or progesterone. The oestrogens induce the development of the female genital tract and progesterone acts on the uterus to permit the implantation of the egg. The principal testicular hormone, or androgen (*C *19 steroids) is testosterone, which is secreted by the interstitial tissue of the testis and controls the morphological and functional development of the male genital tract.*

(24) Free-martin: *the sterile cow which usually develops from the female of two twin calves of different sex. The term may have various origins: probably 'free' comes from the word 'farrow', used to describe an infertile animal in Scotland and England, while 'martin' may come from the Gaelic 'mart', a cow.*

(25) Genital tract: *the different parts of the genital system (genital ducts, accessory glands, copulatory organs).*

(26) Ovotestis: *mixed gonad, formed of ovarian and testicular regions.*

(27) Localised irradiation: *that which is carried out on a closely defined area. It is achieved by canalising beams of rays (*X*-rays or ultra-violet), either by means of metallic tubes which let only narrow beams pass, or by using protective lead shields. The technique of localised irradiation has made it possible to destroy specific regions of embryos and to study the effects of such destruction on development.*

The chemical differentiation of the embryo

Silvio RANZI

It is well known that an adult organism is made up of substances differing from those in the embryo; these in turn differ from those in the egg. So the problem of development has to be examined from a chemical point of view, and a chapter on embryology must show how the various substances forming the adult body appear during growth.

The mineral content in bones is essentially calcium phosphate. Yet Delezenne and Fourneau have proved that a hen's egg contains only 35 milligrams of calcium, whereas up to 141 milligrams can be found in the shell of the egg during embryonic development. According to Ranzi, a similar phenomenon occurs in the development of the cuttlefish. Here the egg contains 0·8 milligrams of inorganic minerals, whereas the embryo contains 3·3 milligrams at full development (fig. 1).

It is interesting to study the fate of the various inorganic elements during the period of development. The bulk of potassium is intracellular, whereas the sodium is largely extracellular. The sodium/potassium ratio increases during the development of the cuttlefish's egg, showing the gradual formation of blood and intracellular liquids. Iron, copper, molybdenum, manganese, all increase during the development of the embryo. This increase must be linked with the synthesis of the metalloproteins[1] containing the element in question which are produced during development. These metalloproteins are essential both to the embryo and to the adult, because of their incorporation in the enzymes.

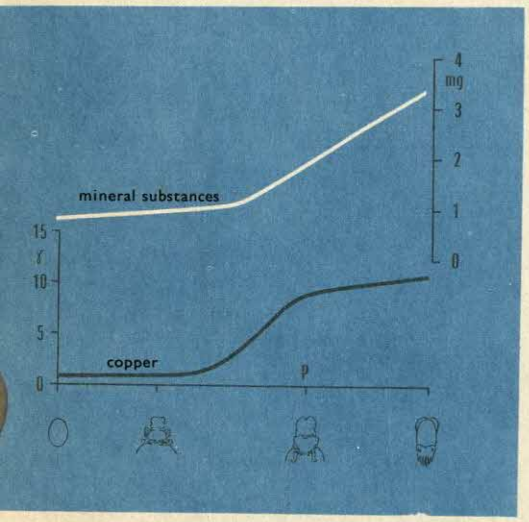


Fig. 1. During the development of a cuttlefish embryo, as with several other animals which lay eggs in salt or fresh water, metal ions are taken from the surrounding water. The increase of mineral substances determined after calcification is shown on the graph; in particular, calcium is absorbed to form the inner shell of the cuttlefish, which develops during the second half of embryonic development. The increase in copper, used in the synthesis of haemocyanin is also shown. P *shows the point where the peroxidase reaction appears, oxidising the benzidine in the circulatory apparatus of the embryo, a reaction which shows up one of the properties of haemocyanin.*

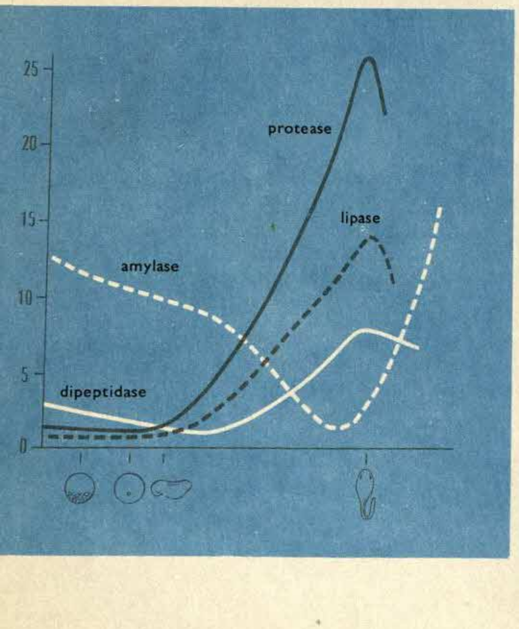


Fig. 2. Variation of the enzyme content feeding the embryos of some species of tail-less amphibians. The graph is based on information from Urbani and shows that after the lipoproteins of the yolk have ceased to be used (in a young tadpole), the enzymatic activities required to digest fats (lipase) and protein substances (protease, dipeptidase) decrease, whilst the amylase enzymes increase.

Several authors, Boell and Urbani amongst others, have studied the increase in enzymes during embryonic development and shown that in some cases the periods of enzyme-increase correspond to particular periods of embryonic development. Fig. 2 shows, for example, that the amounts of proteolytic enzymes (proteases, dipeptases) and lipolytic enzymes (lipases) decrease in tadpoles that have used up all the yolk, which consists mainly of lipoprotein;[2] this occurs because the animal has altered its type of food.

In the case of animals which lay eggs (sea-urchins, birds) and those which give birth to living individuals but which nevertheless have large eggs (sharks, skates, and viviparous reptiles) the newly-hatched egg is less hydrated than the embryo at the end of its development. In these instances development can be seen as a series of metamorphoses in which the vitellus or yolk, containing little water, is transformed into a substance containing more and more water, formed by cellular colloids (mainly protein substances) which are gradually synthetized.

Three ways of studying the formation of substances

There are, in fact, three ways of studying the formation of substances during development. One consists in extracting, separating and analysing by chemical or physico-chemical methods the substance in question and studying the way it changes. Alternatively, we can study its enzymic properties and deduce from these the differences between the proteins; however, if this method is employed, it is necessary to be sure that these differences are independent of the action of any possible inhibition and this in many cases is virtually impossible. Finally, we can study the antigenic properties by immunological methods[3] or use a radioactive tracer.

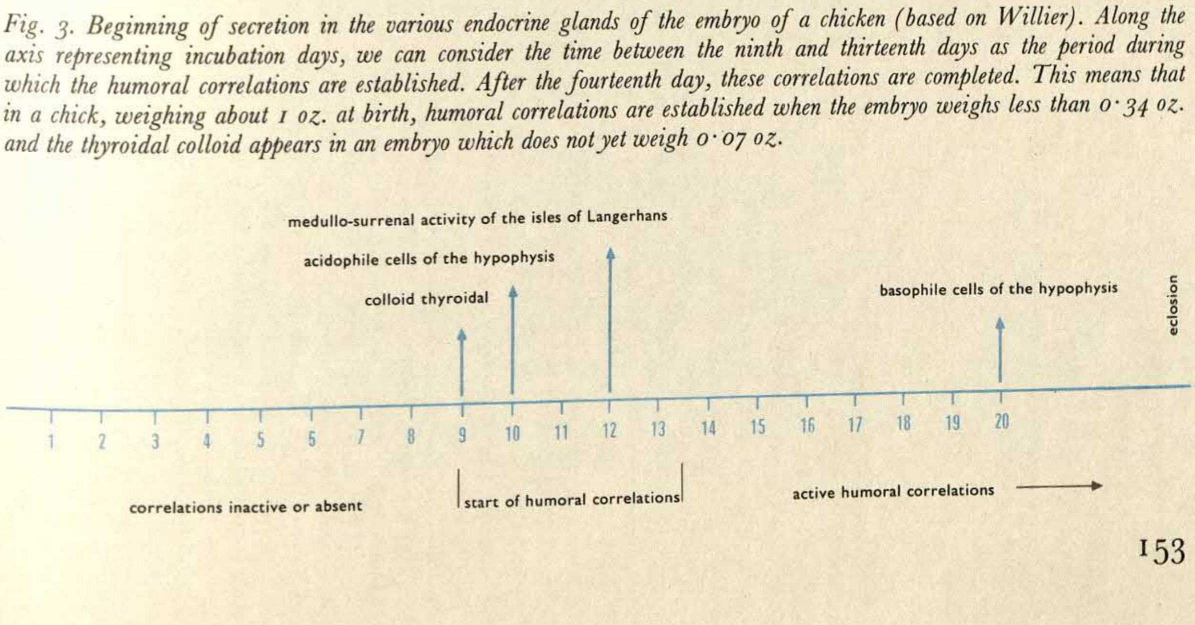
On first approximation it might be thought that cellular differentiation, as seen through the optical microscope by cytochemical methods or by means of an electron microscope, is definitely linked with the synthesis of the corresponding molecules in adult cells. Research workers using the electron microscope have shown that the ribonucleoproteins—that is, molecules containing cytoplasmic nucleic acid—increase when protein synthesis is going on, for example in embryonic cells during cellular differentiation.

The study of nucleic acids by research workers in embryology has demonstrated the gradual synthesis of thymonucleic acid, the nucleic acid of the nucleus, during the process of cell division. There are even some animals in which the formation of the nuclear substances is extremely active from the earliest stages of development. Today, following on the researches of Briggs and King, who, by transplanting the nuclei of various embryonic cells into enucleated eggs at the beginning of development, obtained different results, we cannot be certain that the nuclei formed during these early stages of development are analogous; consequently, they might contain substances identical to those of the nuclei of the adult. So we have no indication as to the exact moment when the characteristic substances of the adult nuclei are formed and when we consider the diagrams of synthesis of nuclear substances, we remain perplexed as to the origins of their differentiation.

The gradual formation of proteins

The gradual formation of proteins has been demonstrated by Heim and Schechtman working on the proteins of the blood serum of a chicken's embryo and using an electrophoretic method (that is, separating them by means of an electric field). After ten days of incubation, the albumin/globulin[5] ratio remains practically constant, but during embryonic development, alpha-globulins decrease, and beta-globulins increase, whilst the gamma-globulins increase during the final embryonic development and following birth. Fibrinogen and prothrombin,[6] on the contrary, appear in the blood only after the twelfth day of incubation. The increase of gamma-globulin after birth is in accordance with what can be observed of the organisms' immunisation reactions. The embryo and the new-born chick cannot form antibodies and, indeed, the technique used in biological laboratories for cultivating bacteria and viruses in chick embryos, makes use of this fact. It is only after birth, with the increase of gamma-globulin, that antibodies can be formed, increasing gradually and reaching a maximum in the fifth week of life. As regards the development of man, Ewerbeck and Levens have shown that in the course of embryonic development the blood serum contains an ever-increasing quantity of proteins, and the albumin/globulin

Fig. 3. Beginning of secretion in the various endocrine glands of the embryo of a chicken (based on Willier). Along the axis representing incubation days, we can consider the time between the ninth and thirteenth days as the period during which the humoral correlations are established. After the fourteenth day, these correlations are completed. This means that in a chick, weighing about 1 oz. at birth, humoral correlations are established when the embryo weighs less than 0·34 oz. and the thyroidal colloid appears in an embryo which does not yet weigh 0·07 oz.



(1) Metalloproteins: *Several enzymes contain in their molecule one or more metal atoms, for example, the oxygen-carriers in the blood: haemoglobin (the red pigment of the red corpuscles of Vertebrates containing iron) and haemocyanin (found in the blood plasma of the lobster, the king-crab and several shellfish, and containing copper). There are peptidases containing manganese, flavoproteins containing molybdenum, others containing iron.*

(2) Lipoproteins: *Protein molecules containing in the molecule a portion formed of lipides are called lipoproteins. Lipovitellin is the typical lipoprotein of egg yolk.*

(3) Immunochemical reaction: *Reaction of a precipitate produced* in vivo *or* in vitro *between serum containing antibodies of the immunised animal and the antigen solution containing the antigens.*

(4) Electrophoresis *(or electrophoretic method): If an electric current passes through a saline solution, the protein substances in this solution move towards the positive pole at different speeds according to the protein. It is therefore possible to separate the various proteins of the blood plasma. In this case (normal plasma) the fraction which moves quickest is made up of albumin; then come the* α *globulin divided into two fractions* α_1 *and* α_2*; then* β *globulin and* γ *globulin, the least mobile, containing antibodies.*

(5) Albumin/globulin ratio: *Albumin, a spherical protein with a rather high molecular weight (between 50,000 and 100,000); globulin, a spherical protein of the blood with a very high molecular weight (from 100,000 to more than a million).*

(6) Fibrinogen: *Fibrillary protein of the blood plasma which becomes an insoluble fibrin when the blood coagulates. Prothrombin: Glycoprotein of the blood plays an important part in the coagulation of the blood. Prothrombin is an essential factor in the formation of thrombin, an enzyme catalysing the transformation of fibrinogen into fibrin.*

ratio increases as development progresses, whilst of the globulins, gamma-globulin increases.. After birth, however, it decreases. According to Bangham, the gamma-globulin of the foetus is not synthesized by the foetus itself but is derived directly from the maternal blood-stream.

Schechtman studied this same problem by an immunological method which is much more sensitive. He observed that the egg yolk contains antigens which react with the antibodies prepared against the serum of the adult. The same phenomenon has been observed in the frog's egg which contains five or six antigens reacting with the antibodies prepared against the maternal serum. However, it has been known for some time from the researches of Lakowski and others, that when eggs are laid there is a special protein in the blood of the hens known as serovitellin, which passes into the growing oocyte to become the characteristic lipovitellin of the egg yolk, enabling the oocytes to grow quickly.

Appearance of haemoglobin, enzymes and hormones

Another question arises in connection with the appearance of the haemoglobin of red cells in the blood of vertebrates. Spirite, utilising the ability of haemoglobin to act as a peroxidase7 oxidising benzidine8 noticed that this enzymatic property appeared in the blood islands9 of the chick embryo and in the two blood islands of amphibian embryos. The embryos of the Cephalopoda, according to Ranzi, demonstrate that copper increases in the blood precisely at the moment when one begins to observe an increase in the reaction of peroxidases. This is due to the reducing properties of haemocyanin, a protein containing copper in its molecule, which occurs in the blood plasma of some large invertebrates and has a similar role to that of the haemoglobin of vertebrates. The absorption of copper and the formation of haemocyanin are, therefore, phenomena which seem to be related, so that the embryo of a cuttle-fish can incorporate copper in an organic molecule.

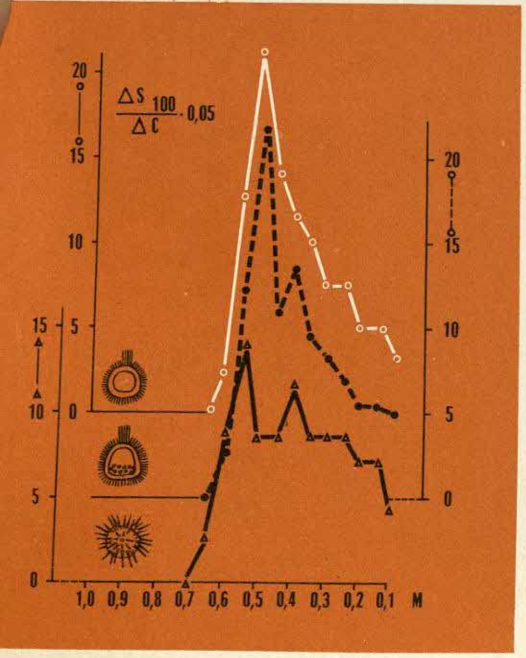


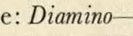
Fig. 4. Synthesis of proteins during the development of the sea-urchin. From a solution of proteins derived from the larva or adult in potassium chloride, one can see that, in the sea-urchin blastula, when the mesenchyme forms, a fraction appears which precipitates when the concentration of potassium chloride is reduced to 0·35 molar. This fraction is shown by the second summit, missing in the diagram of the swimming blastula's precipitate but seen in the diagram of the blastula with mesenchyme, and also in the diagram of the protein precipitate of the adult sea-urchin.

Digestive enzymes are also formed while the embryo is growing. Hydrochloric acid appears in the stomach of the embryo of Selachians, of chickens and of mammals and among the various species of Selachians (shark, skate, etc.) we can follow the digestion within the intestines of the yolk or the uterine fluid. In the stomach of the human embryo, hydrochloric acid and pepsin can be found after the third month, whereas trypsin and the enzymes of the pancreatic juices make their appearance only after the fourth month.

Another question concerns that of the gradual appearance of the various hormones. Cotronei, who has studied this phenomenon in the embryo and larva of various amphibians, found that the humoral correlations were complete by the time the vitellus has been absorbed. At this stage the biochemical individuality of various zoological species is completely established and that is why, in the case of incompatibility, grafts are either rejected or disintegrated. Willier and his colleagues have studied the same phenomena in the development of the chicken's embryo (fig. 3), and in the case of mammals, the classic experiments of Carlson and Drennan have shown, if not the beginnings of insulin secretion, at any rate the moment at which it becomes important. In dogs during the last three weeks of pregnancy, the foetal pancreas secretes so much insulin that if the maternal pancreas is removed during pregnancy no diabetic symptoms appear until birth has taken place. Ukito, carrying out a similar experiment on rabbits in which the thyroid was removed, found that the foetal thyroid is capable of protecting the mother against hypothyroidism only after the ninth day of pregnancy.

It is at a relatively early stage that substances appear which are produced by hereditary factors, diversifying the individuals of a same species or a race. In man, the various blood groups O, A, B and AB are characterised by certain substances, the antigens, found on the surface of the red cells, and A and B antigens appear in embryos that are only a few months old. Erythroblastosis foetalis is a disease brought about by a similar phenomenon, being merely the reaction of a mother, immunised by a first pregnancy against antigen Rh which she does not possess but which the child has inherited from the father.

(7) Peroxidase: *Peroxidase is an enzyme carrying oxygen from a peroxide on to another molecule. The peroxidase which oxidises benzidine can also take oxygen from H_2O_2 and connect it to the benzidine which, thus oxidised, takes on a bluish colour.*

(8) Benzidine: *Diamino—4·4'—diphenyl:* 

(9) Blood islands: *The blood islands form the first rudiments of vessels and blood. A mass of cells drawn from the visceral mesoblast which become hollow in such a way that the peripherical cells become arteries and the central ones become blood cells.*

(10) Actomyosin: *The muscular fibril consists of two proteins: myosin and actine. These two proteins join together and form actomyosin.*

(11) *Information on embryonic stages: In the first stage of the development of the embryo of the chick, a line develops which represents the axis of the body of the chick to be. This line is called the primitive streak and is the area where the cells of the mesoderm will be formed. Later, the anterior end of the primitive streak becomes the head fold. In the development of the frog, the stage of the vitelline plug corres-*

Continued on next page

When do the various substances become differentiated?

If we observe the differentiation of the various organs and tissues, it becomes apparent that, generally speaking, a considerable quantity of cells or non-differentiated intercellular substances are formed initially and that the characteristic substances are only differentiated later. Thus, as the mitochondria become differentiated within the cells, enzymes of cellular respiration, the cytochromoxidases and succinodehydrogenases, make their appearance attached to the membranes of the mitochondria. Studying the muscular apparatus of a chicken's embryo, Hermann and his colleagues noted that increase in size is due mainly to an increase in the space occupied by the cells and an increase of actomyosin,[10] the specific protein of muscles. As soon as muscular contraction commences, so does the activity of adenosinetriphosphate, the enzyme which plays a major part in muscular activity.

Ranzi and Citterio, studying the properties of the precipitate of various proteins which can be extracted during the development of sea-urchins, discovered that during the formation of the mesenchyme in a swimming blastula, proteins having the characteristics of the proteins in adults are synthesized (figs. 4, 5 and 6). By an immunological method they succeeded in proving that at least four different adult antigens can be shown to exist at this stage. This observation tallies completely with previous research by the Runnström school, which showed that the period of mesenchyme formation is a period of intense protein synthesis.

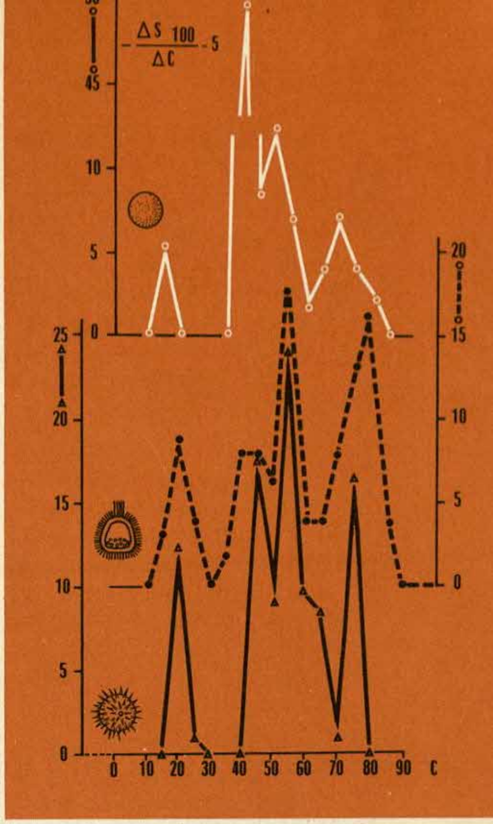


Fig. 5. Synthesis of proteins during the development of the sea-urchin. Diagrams of the protein precipitates obtained by adding ammonium sulphate to proteins dissolved in a highly alkaline solution of potassium chloride; the ordinates show the quantity of proteins which precipitate; the abscissae show the concentration C *of ammonium sulphate. The intermediate diagram shows the precipitate of proteins of the blastula with mesenchyme, the first three peaks corresponding to those in the adult (bottom diagram); the diagram of the egg (above) is completely different; the proteins of the blastula when the mesenchyme is formed have the same properties as proteins in the adult as far as precipitates are concerned; these are not found in either the egg or the blastula before the mesenchyme is formed. Formed from the union of two cells, the egg and the spermatozoon; the result of a complex series of differentiations—the human being eighty days after conception. (Mag. × 4 approx. Photo Lod).*

Such examples could be multiplied. In certain cases, which, however, have been studied only by immunological methods, antigens appear at a much earlier stage than might be expected. Ebert noted the appearance of cardiac myosin and actine in the chicken at the phase of the first cleavage and the appearance of the cephalic extremity respectively;[11] Ranzi and Citterio found that extracts of frog embryo at the stage of the vitelline plug react with antimyosin and antiactin sera. Comparing the studies of these two embryonic stages, observed facts were identical: the

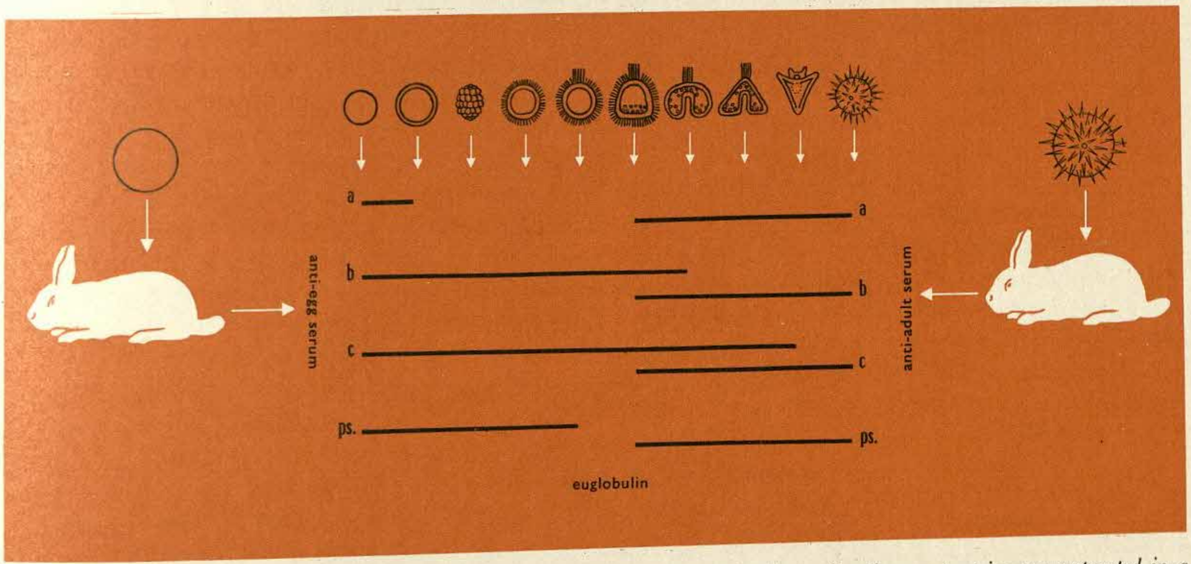


Fig. 6. Synthesis of proteins during the development of the sea-urchin. Behaviour of antigens: proteins are extracted in a solution of potassium chloride and fractionated by dilution into euglobulin a, euglobulin b, euglobulin c and pseudoglobulin ps. The horizontal lines represent precipitates obtained with the various fractions taken during the stages of development drawn above and obtained, for the left-hand lines, with an anti-egg serum, and for the right-hand lines, with an anti-adult serum.

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ponds to an advanced stage of gastrulation. If one studies the formation of the mesoderm, one can say that the stage of the vitelline plug corresponds to that of the primitive streak for, in both cases, the mesodermal cells place themselves between the ectoderm and the endoderm without having yet reached their final position.

(12) Globin: *Protein substance in the molecule of haemoglobin.*

(13) Cooley's disease: *This disease, also called 'Mediterranean disease' is a form of anaemia shown in the first year of life by reduction in the number and a change in the shape of the red corpuscles; there are also alterations in the skeleton, especially the bones of the skull-cap and the long bones. Subjects suffering from this illness do not generally survive puberty.*

(14) Regeneration of the crystalline lens: *In adult salamanders, if the lens of the eye is removed, it grows again from the iris by a process known some time ago and which has been studied experimentally in various ways.*

antigens myosin and actin are present in both vertebrates at the time when the mesoderm is being displaced, that is before the differentiation of the somites. It has yet to be ascertained whether it is myosin or actin which appears at this early stage or merely reactive groups of these proteins.

However, the formation of the various substances during development is not always as obvious as those which we have described. Thus the haemoglobin which first appears in vertebrates is not the same as the haemoglobin of the adult form. We know that there is, in the human embryo, a foetal haemoglobin still present in the new-born child before it is replaced by adult haemoglobin. The difference between the two haemoglobins is in the proportion of globin[12] in the molecule, and from the physico-chemical point of view the two haemoglobins differ in their affinity to oxygen, their resistance to denaturation, and their electrophoretic mobility. Indeed, there is a hereditary disease in man known as Cooley's anaemia[13] in which the foetal haemoglobin remains in the adult. The study of the haemoglobins in the development of various animals has been thoroughly investigated. Using comparative criteria, Chieffi and his colleagues found that the haemoglobins of various lower vertebrates (lamprey, eel, frog) differ before and after metamorphosis. In this series of researches the differences were shown up mainly by means of electrophoretic mobility.

Metamorphosis and differentiation

Amongst the amphibians several substances change during metamorphosis. The most striking example is perhaps that studied by Wald relating to the photosensitive substance of the retinal rod, which is porphyropsin in the tadpole and rhodopsin in the adult frog. Apparently prior to metamorphosis vitamin A2 is synthesized by the liver—this being one of the components of the porphyropsin molecule—whereas after metamorphosis, vitamin A1 is synthesized, which is one of the components of rhodopsin. A similar phenomenon takes place, according to Burcke and his colleagues, in the development of some of the organs of the chicken, in which during the early stages certain characteristic antigens appear in such organs as the crystalline lens of the eye and the brain. These disappear as development progresses and the characteristic antigens of the adult soon begin to multiply. This observation agrees with those made by Markert studying the synthesis of the retina in the embryo of the chicken. By feeding this primitive organ with glucose marked by carbon 14 it can be observed that, at successive stages, the glucose is used to form ever-increasing quantities of glutamic and aspartic acid, whilst their synthesis of other substances gradually decreases. Thus synthetic activity alters during development.

Similar observations were made by Ranzi and his colleagues in their research on antigens in two kinds of Italian toad (*Bufo bufo* and *Bufo viridis*) and their hybrids. There exists in both *Bufo bufo* and *Bufo viridis* an antigen or group of antigens which appear during gastrulation, originating from a specific reactive group. This same molecule, after the nervous system has been formed, presents a second reactive group which reacts with the antiserum of the other kind. Here again we have a molecule which is different in the young embryo and in the embryo at a later stage.

So it can be asserted that specific proteins appear at various stages in the various cells or in the intracellular liquids and substances. These proteins often have characteristics different from those in the adult, even if the substances are so similar that the differences have only recently been discovered.

Localisation of antigens in the embryo

There is also another series of facts: when antigens begin to appear, they are often found to be more wide-spread than is consistent with the rudiment of the organ concerned. Such, for example, is the case of the cardiac myosin studied by Ebert: the reaction of the precipitate with anti-myosin serum envelops all the blastoderm and not only that part of it corresponding to the heart rudiment. Only when the cephalic extremity is well differentiated do the antigens of the cardiac myosin become restricted within the rudimentary heart.

Van Doorenmaalen's observations lead to similar general conclusions. This author found some of the antigens to the crystalline lens in the iris. Little can be said as yet about these observations, but it would be most interesting to relate them on the one hand to the idea of the antigen localised from the moment of fertilisation (in the case of cardiac myosin) and on the other to the possibility of regeneration[14] of the crystalline lens from the iris, which exists in several amphibians.

HISTORICAL NOTES

1866
In an inaugural lecture given at Dorpat University, E. KORBER showed that human embryonic haemoglobin is more easily altered than adult haemoglobin.

1885
W. PREYER published his book Spezielle Physiologie des Embryos *(translated into French by M. Wiet and published in Paris in 1887). This book summarises all that was known at that time about the subject of this chapter.*

1887
C. B. DAVENPORT proved that the increase in the weight of an embryo is linked with the amount of water absorbed by the embryo from the medium in which it is developing.

1903
P. UHLENHUTH discovered that the lens possesses organo-specific antigens.

1911
A. J. CARLSON and F. M. DRENNAN discovered that there was no glycaemia in a bitch whose pancreas has been removed during the last weeks of pregnancy; diabetes develops immediately after she gives birth, which proves the passage of foetal hormones to the mother.

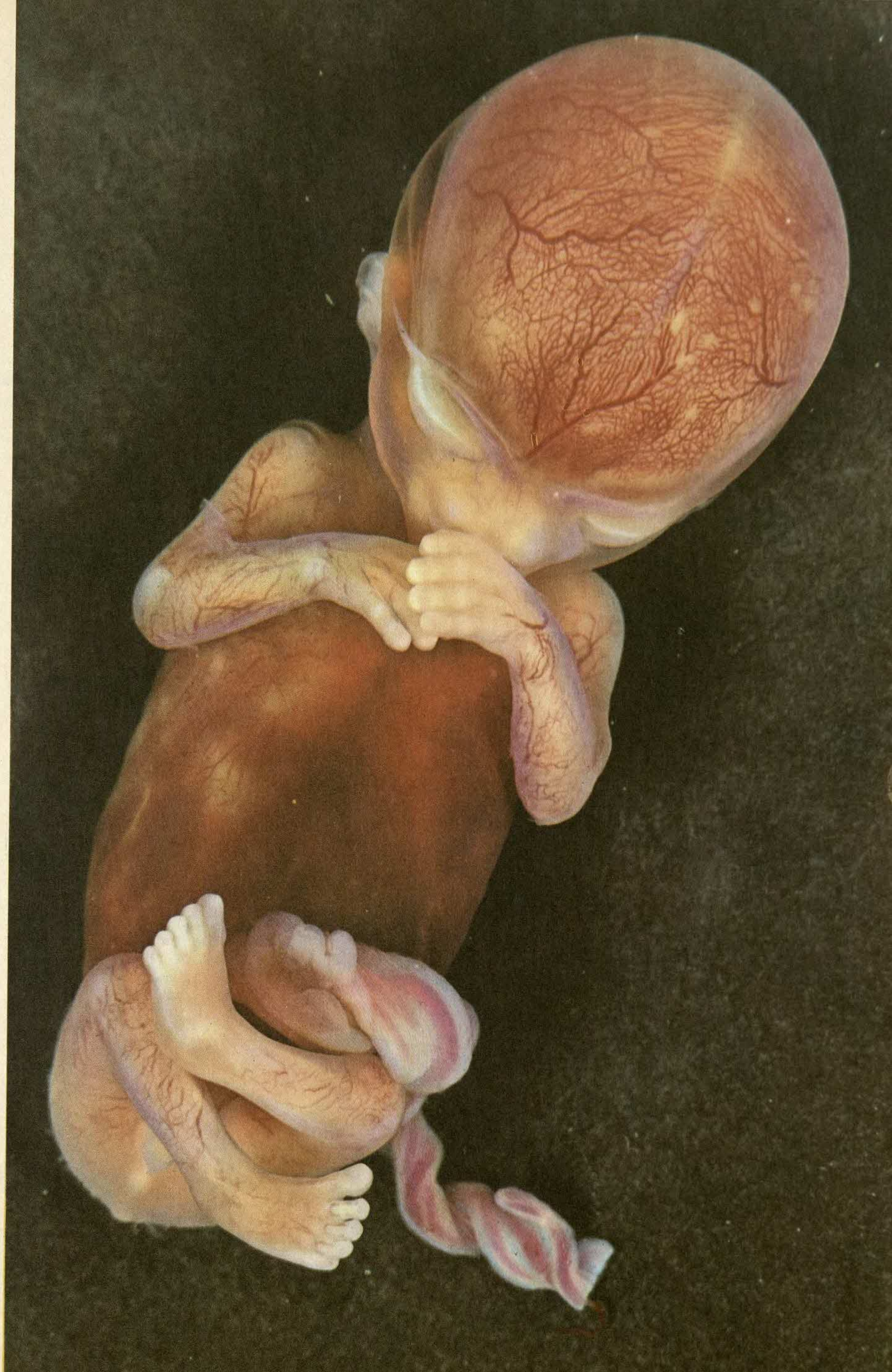
1931
J. NEEDHAM published his Chemical Embryology.

1941
P. LEVINE and his colleagues interpreted the foetal erythroblast already described in 1932 by L. K. DIAMMOND, K. D. BLACKFAN and J. M. BATY, as an illness due to maternal antibodies against something the embryo had inherited from the father.

1942
A. H. COONS, H. J. CREECH, R. N. JONES and R. BERLINER perfected the fluorescent anbibody method, which was then developed with KAPLAN (1950). Thus localising of antigens in the cells and tissues became possible.

1952
R. M. CLAYTON presented to the International Institute of Embryology the results of his research work, subsequently published in 1953, on the appearance of various antigens in the germ layers and the primitive organs of the embryo of the salamander.

1955
J. EBERT and S. RANZI and their colleagues, working individually and by different methods on hens and frogs, discovered the antigens myosin and actin, which can be discerned long before the individualization of somites and form the rudiments of the muscular apparatus.



Formed from the union of two cells, the spermatazoon and the ovum—the result of a complex series of differentiations: the human embryo 80 days after conception. (Mag. × 4) (Photo Lod).

Conclusions

Etienne WOLFF

It is of course premature to draw conclusions from partial knowledge. Neither the present state of science nor the glimpses shown by these studies enable us to deduce categorical laws, to enclose life within rigid equations. Knowledge would perhaps lose some of its charm if we could close the book, knowing that all there was to say had been said.

But we can make some remarks which force themselves upon those who explore the various fields of biology, and in particular upon those who make experiments. First, that living matter is both malleable and stable. It can be cut, grafted, added to, and taken from rudiments of organs of young embryos; organisms can be submitted to abnormal conditions, to physical and chemical factors which stimulate or cause harm.

Yet what transformations are finally induced, save for a few anomalies or mutilations, a few changes of detail, stimulating or inhibiting innate characteristics? Often the intervention will have no long-lasting consequences, because the organism possesses a remarkable aptitude for self-regulation, tending to establish its integrity anew, or to limit the damage caused, by embryonic control at the early stages of development, regeneration or healing in the adult, and maintenance of the internal environment. Consider, for example, the incredible amount of medicine, drugs, poisons and toxins absorbed during the life of a modern, normal man, without greatly modifying the working of his organism either for better or worse.

And when some large-scale alteration has been achieved in an embryo resulting, for example, in a monster, the limit of the species will not have been transcended, the plan of organisation will not have been altered, but something will have been made out of this plan. A giant chicken remains a chicken, a giant pig remains a pig. Etienne Geoffroy-Saint-Hilaire's one-time dream, that by some action on the embryo one species could be transformed into another, has not yet come true. For species are always stable within normal conditions and the plan of organisation imposes itself in an almost dictatorial fashion. No doubt evolution has come to pass through countless alterations in the species, but, in nature, on the scale of human life or historical eras, no great alterations occur. Transmutations, that is one species becoming another, are rare. By means of certain external factors, such as x-rays or chemical substances, some mutations have been induced, that is, some racial alterations, but only in an arbitrary and unpredictable manner and for a limited number of animal or vegetable species. Much is expected from the deoxyribonucleic acids which, in bacteria and perhaps in ducks, have produced complete changes of type. But these, it must be admitted, are very small alterations. From a theoretical point of view and on the scale of geological eras, the exceptional importance of these alterations must not be under-estimated: but they do not belie, in the eyes of mortal man, and within the framework of a short-lived experiment, the great stability of living matter.

Another impression which the biologist acquires from his efforts to understand is that although the analysis of vital phenomena has made great progress, their synthesis has not. Living matter can be divided into its simple components, mechanisms can be dismantled, organisms can be sectioned into organs, tissues and cells, but from the separate elements, be they the chemical components of the cytoplasm or the structural elements of the living being, it is not possible to reconstitute a whole. It is certainly remarkable that it has proved possible to cultivate tissues and cells outside the body, to watch them grow and multiply for long periods or even indefinitely on suitable media. Is it not incredible that it has been possible to keep alive for several scores of years the cells of an organism which, if they had remained in the body, would have long since died? Is it not even more incredible to be able to cultivate, in laboratories all over the world, the cancerous growths taken from a human being whom they have in fact killed, and to think that these cells can continue to proliferate indefinitely?

But having reached the limits of analysis, the biologist is generally unable to carry out the synthesis of that which he has dissociated. If, sometimes, he has advanced along the path of chemical synthesis of the components of proteins, if he has re-formed accidentally some tissues from their component parts, he is still far from being able to recreate living matter or to make up an organism from its dissociated elements. In the present state of scientific knowledge, one can say that one of the characteristics of life is the impossibility to. synthesize it.

Another characteristic of living beings is the extraordinary precision of the mechanism of growth, of the integration within adult organisms. Everything is ready when required and where required, everything forms a harmonious whole with no gaps, and one cannot help admiring this complexity which is apparently not directed by anything, but controls itself by self-coordination of the various mechanisms, by the action and reaction of one organ on another, by the interrelation of the various functions, by these reciprocal phenomena which specialists call 'feed-back'. Science has just begun to clarify these mechanisms and their connections.

One cannot help noting how the commentator never spares his admiration whenever he tries to describe the mechanisms of life. It is quite certain that organisms have many more resources and much more pliable connections than those of any machine. But the possibilities of the organism must not be overestimated. If it often triumphs over dangers during growth, if it adapts itself to many unfavourable circumstances during its life, it has its limitations and can only adapt itself within them. Even if he were starving, man could not digest wood or cellulose, which are readily assimilated by other organisms. One admires the marvellous way in which many species adapt themselves to their environments, but one must also acknowledge that many lead an appalling existence. We may admire the graceful and easy way in which swans, ducks and penguins swim, but let us also recognise that, on terra firma, they walk like cripples.

The organism is a very complex chemical laboratory; indeed, each cell represents such a laboratory. Every instant substances, in themselves highly complex, are created and destroyed, defying all attempts at synthesis or analysis, such as can be carried out in a chemical laboratory. And when man does succeed in imitating life, by a synthesis of one of the many organic combinations he can now prepare, what differences there are in the means employed. The chemist generally uses powerful means, acids of varying strength, strong alkalies, and the reactions he induces require a high temperature. In the organism, everything occurs at body temperature, in the presence of those biological catalysts the enzymes and diastases. At each stage of a synthesis or a degradation, one of these substances is present, connected by another one which pushes the reaction further forward. Powerful compounds can also intervene during such processes; for example, such substances as phosphoric acid, hydrochloric acid, and formalin. But these are combined as soon as they form, and their appearance is so fleeting that, like many of the products of intermediate stages, it requires great ingenuity to detect them.

Apart from the general laboratories of the organism, such as the ordinary cells, there are also highly specialised laboratories in certain organs—the endocrine glands, for example—where substances are formed which are used by other organs for purposes which have nothing to do with the conservation of the organ that produces them.

All this highly complex specialised chemistry is only just beginning to be understood. It follows a fixed order, without any violence, by methods which sometimes still seem baffling. That which biochemists and biophysicists of great learning have spent years to find out, using their intelligence to the full, that which still remains hidden from our methods of investigation, appearing from our intellectual approach to be vastly complicated and almost incredibly difficult, is done quite smoothly by life itself. The single cell of a modest earthworm knows more on this subject than the most brilliant human intellect, defying the accumulated knowledge of many years.[1] Let us therefore recognise the technical superiority of organic processes over conscious understanding, but also let us not forget that each new discovery made by the intellect gives us more hold over the pageant of vital phenomena.

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(1) *This is what, in modern 'cybernetic' terminology is called the 'amount of information' contained in the genes of an organism.*

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